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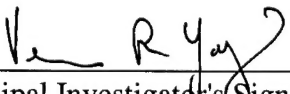
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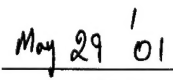
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## Table of Contents

<b>INTRODUCTION .....</b>	<b>5</b>
<b>HYPOTHESIS TESTED .....</b>	<b>14</b>
<b>TECHNICAL OBJECTIVES .....</b>	<b>14</b>
<b>METHODS .....</b>	<b>15</b>
SUBJECTS .....	15
EXPERIMENTAL DESIGNS .....	17
<b>SHORT-TERM, COMPLETE FASTING STUDIES: .....</b>	<b>17</b>
APPROACH .....	17
MAJOR FINDINGS .....	19
SUMMARY AND CONCLUSIONS.....	20
<b>LONGER-TERM PARTIAL CALORIC RESTRICTION STUDIES .....</b>	<b>20</b>
APPROACH .....	20
STUDY DURATION AND PHASES .....	21
DESIGN OF 24 HOUR TRACER INFUSION, SUBSTRATE KINETIC STUDIES .....	21
BLOOD SAMPLING .....	23
BREATH SAMPLING FOR $^{13}\text{CO}_2$ ANALYSIS .....	23
WINGATE TEST .....	24
DUAL ENERGY X-RAY ABSORPTIOMETRY .....	24
STRENGTH AND ENDURANCE PERFORMANCE TESTS.....	24
VERTICAL JUMP POWER .....	25

TESTS OF MENTAL PERFORMANCE .....	25
ACTIVITY.....	26
WILKINSON AUDITORY VIGILANCE TASK .....	26
FOUR-CHOICE REACTION TIME TASK .....	27
CALCULATION PERFORMANCE TASK.....	27
TRACKING TASK .....	27
PROFILE OF MOODS STATES (POMS) .....	28
<b>SAMPLE ANALYSIS .....</b>	<b>28</b>
ISOTOPIC AND NON-ISOTOPIC ANALYSES IN PLASMA .....	28
<b>EVALUATION OF PRIMARY DATA.....</b>	<b>29</b>
<b>RESULTS OF THE LONGER-TERM PARTIAL ENERGY RESTRICTION IN MEN AND WOMEN .....</b>	<b>30</b>
BODY COMPOSITION .....	30
DIETARY CHARACTERISTICS.....	31
<b>METABOLIC PARAMETERS .....</b>	<b>32</b>
LEUCINE OXIDATION .....	32
<i>24 h Leucine Oxidation.....</i>	<i>32</i>
<i>Leucine Oxidation during Exercise .....</i>	<i>32</i>
GLUCOSE PRODUCTION.....	33
<i>Total glucose production (baseline, exercise and recovery).....</i>	<i>33</i>
<i>Glucose production during exercise .....</i>	<i>34</i>
GLYCEROL KINETICS .....	34

<i>Total glycerol Ra (baseline, exercise and recovery)</i> .....	34
<i>Glycerol Ra during exercise</i> .....	35
PALMITATE KINETICS.....	35
UREA PRODUCTION.....	35
TOTAL ENERGY EXPENDITURE.....	36
PHYSICAL PERFORMANCE .....	37
MENTAL PERFORMANCE .....	37
<b>REFERENCES</b> .....	<b>40</b>

## **Introduction**

### **(i) The Military Significance**

Weight loss, an index of malnutrition, occurred in trainees of the US Ranger Training Brigade. This weight loss can amount to about 15.6% of initial body weight or somewhat less (12.6%) when a nutrition intervention, that includes a 470 Kcal/day supplement is provided together with additional protein (1,2). While this nutritional supplement might reduce the severity of the weight loss, the latter was highly variable among the subjects; for some it was 15-18% body weight. According to Kinney (3) the caloric equivalent of the weight loss amounted to about 970 Kcal/day in the Ranger II study and it was also concluded that "Ranger training did not change the pattern of tissue loss seen in studies of partial starvation without such heavy physical exertion." This observation is important since it means that studies in volunteer subjects who are not enrolled in the military might serve as a model to help clarify the metabolic and physiological processes that are responsible for these changes in body weight and composition (with fat accounting for 61% of the total weight loss). Furthermore, the functional and behavioral significance of these weight changes are still poorly understood, although there was a 23% reduction in lifting strength, impaired mental performance, reduced immune function and increased infection rates indicating increased susceptibility (2).

A further issue of considerable strategic importance to the Department of Defense relates to the increasing participation of women in all aspects of military service. According to King et al (4) there are now over 1/4 million female members on active duty in the US military services, yet adequate information is lacking on the nutritional status of women serving in the Armed Forces. Furthermore, King et al (4) conclude that while the nutritional problems of military women are similar to their physically active civilian counterparts involved in sports, the nutritional problems of military women may be exacerbated by the need to meet military body-weight and percent body-fat standards. Therefore, it is critical that new knowledge be forthcoming on the metabolic and nutritional characteristics of military women in order to develop sound and effective nutrition policies and programs designed to maintain health and achieve performance goals of this growing segment of the US military.

**(ii) Energy and protein metabolism in reference to females**

Sufficient data existed to suggest it would be highly desirable to conduct, within the context of the nutritional/metabolic/ clinical changes observed in the previous male, Ranger studies (1,2), a detailed series of investigations on the metabolic responses, and capacity to adapt, to conditions of a dietary energy deficit in women and to compare these with those that occur in their male counterparts.

Briefly, the reasoning behind this was based on the following published observations. Thus, there are changes in energy metabolism and dietary macronutrient intake levels (5) during different phases of the menstrual cycle. The metabolic rate during rest (RMR) and sleep (SMR) is generally believed to represent the cost of

maintenance and restoration of body tissue and energy stores. The contribution of RMR to total energy expenditure (TEE) is generally 65-75% in healthy, mildly active subjects. Of particular relevance here is the evidence that in women changes of metabolic rate under basal conditions (BMR), at rest or sleep, show a periodicity which coincides with the different stages of the menstrual cycle (6-9). A decrease in BMR has been observed during menstruation, falling to the lowest point approximately 1 week before ovulation. Then BMR rises during the luteal phase until the next menstrual period. The increase in the post-ovulatory phase is ~ 8%, on average (7,8).

Since the contribution of BMR to TEE is high, we would expect 24-hour energy expenditure to be increased in luteal phase. Indeed, 24-hour energy expenditure in the follicular and luteal phase was evaluated in standardized living conditions, so that subjects had the same activity schedule and food intake during both measurements. An average increase of 9% was observed 2 weeks after ovulation (10). This finding has been confirmed in a more recent study (9), but the increase was smaller, although statistically significant. The thermogenic effect of progesterone also has been assessed (11), suggesting that the moderately increased level of progesterone secreted by the corpus luteus in the post-ovulatory phase is a potential cause for the elevated metabolic rate.

At present, it is not known if the increase in energy expenditure observed in the post-ovulatory phase is due to a relatively higher utilization of fat, glucose, or proteins or if the oxidation of all substrates is increased *in toto*. Some information was provided by the study of Bisdee et al (7) which showed that carbohydrate oxidation tended to be lower and fat oxidation to be higher in the early follicular compared with the luteal phase.

Also, glucose metabolism has been extensively investigated but no major differences have been observed in glucose production (12,13), oxidation (13), or insulin sensitivity (13,14).

Changes in lipid metabolism during the menstrual cycle have received little attention; by measuring the rate of free fatty acid (FFA) appearance (Ra) into the bloodstream, as index of lipolysis, no differences were observed between the follicular and luteal phases (15). However, it is well known that FFA Ra represents a combination of two opposite processes: lipolysis and reesterification to triglycerides. It is quite possible that lipolysis as well as reesterification were higher in one of the two phases whereas FFA Ra did not increase. The FFA-triglyceride cycle is a substrate cycle, that does not result in any increase in the net flux of substrate but requires energy and produces heat (16). From metabolic studies performed in men, the energy cost of the FFA-triglyceride cycle appears to account for a relatively small proportion of RMR (17). However, there is no information about the role of this substrate cycle in relation to the observed periodic changes in energy expenditure in women.

There are contrasting findings concerning the impact of the menstrual cycle on protein metabolism. No statistically significant differences were observed in whole-body protein turnover during the menstrual cycle when a single oral dose of  $^{15}\text{N}$ -glycine was administered and urinary ammonia, as the end product was measured (18). On the other hand, a biphasic cycle in urinary nitrogen has been observed (19); the lowest nitrogen excretion occurred at the time of ovulation, tending to increase during the following week and falling again just before, or together with, the onset of menstruation. Interestingly, in these same women, the nadir of the RMR curve preceded the nadir of

the nitrogen excretion curve by about 7-10 days. Moreover, during the luteal phase the peak of RMR corresponded to the peak of nitrogen excretion and during the follicular phase the nadir of RMR corresponded to the second peak of the biphasic nitrogen excretion curve. These findings are difficult to explain because detailed information is lacking about energy and amino acid substrate utilization in the female under varying physiological states.

There is general agreement in the literature about the relatively greater precision and sensitivity of the measurement of whole-body leucine oxidation, as compared with the measurement and interpretation of urinary nitrogen excretion data. This tracer method has not been used to evaluate changes in protein oxidation during the menstrual cycle. Thus, to develop a more complete picture of the metabolic response pattern of nutritional stress in women, it would be useful to evaluate whether menstrual cycle-dependent changes in protein metabolism (and also lipid and carbohydrate utilization) modulate energy expenditure and the capacity of women to adapt to a dietary energy deficit and/or food restriction.

In our research program we assessed lipolysis by infusing [ $^2\text{H}_5$ ]-glycerol and measuring glycerol turnover; this approach offers a more precise index of lipolysis than the determination of FFA turnover per se (20,21). In fact, glycerol formed during lipolysis cannot be reesterified to a significant extent in human adipose tissue, and so it is released into the bloodstream (20). Free fatty acid release was measured by infusion [ $2,2\text{-}^2\text{H}_2$ ]-palmitate (21). The difference between lipolysis and FFA release is a measure of FFA intracellular reesterification; the difference between FFA release and FFA oxidation (from indirect calorimetry) will be considered equal to FFA peripheral (hepatic)

reesterification. Glucose production is measured using [6,6-<sup>2</sup>H]glucose as a tracer (20) and carbohydrate oxidation also was measured by indirect calorimetry (20). Protein turnover and oxidation was measured by [1-<sup>13</sup>C]leucine infusion, as described in detail below.

### **(iii) Metabolic response to a complete fast**

The metabolic response to fasting and starvation is characterized by an adaptive shift from a carbohydrate-based fuel economy to one of fat utilization (22). Mobilization of FFA from adipose tissue stores provides the majority of energy needs of peripheral tissue during fasting, sparing glucose for utilization by glucose-requiring tissues, such as the brain (23) and erythrocytes. Several findings indicate that protein sparing in skeletal muscle during prolonged starvation depends on the availability of lipid fuels (24,25).

The effect of fasting and starvation on fuel metabolism has been quantified in men.

Proteolysis and protein oxidation increases by ~40-50% after a 60 h (17) to 72 h fast (26). Glucose production decreases ~25-30% (17,26,27) together with a reduced (~85%) rate of glucose oxidation (17). Adipose tissue lipolysis increases ~2.5 fold (28) providing substrate for the nearly doubled rate of fat oxidation (17). FFA reesterification increases ~3 fold after a 60-hr fast (17) and ~ 6 fold after a 4 day period of starvation (28), representing 2.5% of RMR. The increased activity of the FFA-triglyceride cycle might appear to be paradoxical, given the increased demand of FFA for oxidation by peripheral tissues. However, after prolonged starvation, if no reesterification occurred,

the calculated rate of triglyceride energy mobilization would be more than twice that required for energy expenditure (17,28). The FFA that are mobilized in excess of energy requirements, are recycled back to triglycerides, and at the same time, due to the accelerated lipolytic rate, glycerol is maximally made available for gluconeogenesis. Overall, however, RMR apparently changes little between an overnight and 60-hr fast, because the increase in fat oxidation, and to a lesser extent protein oxidation, compensates for the decline in carbohydrate oxidation with fasting (17).

The foregoing summary account of the metabolic response to starvation is quite tentative - many studies have involved obese rather than individuals of normal weight and body composition and, to date, almost all the subjects studied were male. No comparable quantitative metabolic data are available in women with body weight and fat standards to meet military guidelines. It would be of great physiologic and practical interest to evaluate possible differences between men and women, with these body characteristics, with respect to their metabolic adaptations to short-term fasts (or prolonged but not a profound hypocaloric deprivation).

Sex-related differences in plasma concentrations of metabolic fuels have been evaluated, to a limited extent, in fasting. After 3 days of starvation, women show lower glucose concentrations and either FFA and ketone body levels than do men (29-31). However, no difference have, so far, been observed between the phases of menstrual cycle (29), under these conditions. The possible relation between hormones and blood fuel concentrations was also evaluated. Plasma insulin levels did not differ between men and women but plasma glucagon levels were almost double in women as compared with men, in two studies (29,30), while not different in another (31).

The role of sex hormones also has been evaluated to a limited extent. Estradiol and progesterone administered to adult female rats produce increased ketonemia and hypoalalinemia during fasting, relative to untreated control animals (32). Also, the effect of an oral estro-progestinic contraceptive on substrate concentration during an 84-h4 period of starvation was evaluated in pre-menopausal women (32). No differences were observed when compared with untreated women, with the exception that for the 36 hr interval ketone levels were higher in women receiving the oral contraceptives.

It is not clear why women develop lower blood glucose concentrations than do men after a fast. Hepatic glucose production could be decreased to a greater extent in women than in men; total amino acid and gluconeogenic amino acid concentrations were higher in men than in women, during the first 36 hrs of starvation (31). It is also possible that fasting-induced insulin resistance is less in women than in men, resulting in a higher rate of glucose utilization. The higher FFA concentrations could be the result of higher lipolysis, perhaps a reflection of the higher percentage of body fat mass in women than in men. A reduced FFA reesterification might also be responsible for the greater FFA availability in women. The higher ketone body concentrations observed in women support the hypothesis that FFA are mainly oxidized rather than reesterified. On the one hand, it is possible that the concentrations of FFA and ketones are higher in order to compensate for the lower blood glucose levels. On the other hand, it is well-known that hyperketonemia reduces plasma concentrations of alanine (33), a key gluconeogenic substrate. Consequently, the lower blood glucose level in women might just be a consequence of hyperketonemia-induced inhibition of gluconeogenesis.

There is no information regarding whole-body or organ protein breakdown and oxidation in response to starvation in women. Finally, however and of considerable relevance for the present proposal, is the observation by Carraro and Wolfe (34) that a high-protein diet preceding a 3-day period of fasting in men exerted a protein-sparing effect; those investigators suggest a generous protein intake might be beneficial for those who are exposed to nutritional stress. It would be pertinent to know (a) whether their findings can be confirmed and (b) the nature of the comparative responses in women and men when a diet high in proteins precedes the stress created by a 3 day fast.

Hence, from the above, an aim of these investigations was to test the following hypotheses: after starvation 1) hepatic glucose production is lower in women; 2) lipolysis and lipid oxidation increases more than in men; 3) FFA are reesterified to a greater extent in women than in men; 4) protein catabolism is blunted in women compared to men; 5) a high protein diet prior to a period of short-term starvation alters the metabolic response and blunts the loss of body nitrogen.

#### **(iv) Summary of background review**

Current knowledge concerning the metabolic response of women of normal body composition to periods of nutritional stress, as created by short-term starvation or more prolonged hypocaloric intakes, is limited. Most of the available quantitative metabolic data are based on studies in obese men and women or in non-obese men. There are reasons to anticipate that women might respond differently than men under these nutritional-stress conditions. Since negative body energy balance

may well develop in military women under various operational situations and this metabolic state can compromise the health and performance of military personnel, it is now critical that relevant data be generated that can serve as a basis for establishing a sound and effective food doctrine” for assuring the health and maintaining the performance of military women.

### **Hypothesis tested**

The hypothesis that we have explored is as follows:

The metabolic response to a 3-day fast and to a 3-week period of hypocaloric intake differs between healthy, military-eligible women and men. This gender-dependent response leads to a more favorable maintenance of lean body mass in the female and, in consequence, there is a lowered risk of untoward effects on health and physiologic function in women than in men.

The data generated from the above hypothesis-based investigation was anticipated to provide an essential scientific database for purposes of improving the design and implementation of programs aimed at the nutritional support and maintenance of the nutritional status of women in the military.

### **Technical Objectives**

This investigation had the following major technical objectives:

(a) To compare and contrast the progressive (temporal) alterations in lipid, glucose and protein metabolism during short-term fasting in young women and men, who met the military body-weight and percent body-fat standards.

(b) To compare and contrast

- i. changes in body composition
- ii. mental and physical performance
- iii. kinetics of major energy-yielding substrates and protein metabolism,

under resting conditions and during moderate exercise, during a three-week period of hypocaloric feeding. An energy deficit of about 900 Kcals/day was used for this purpose. Kinetic and metabolic studies included feasible and novel 24-hour stable isotope tracer-indirect calorimetry protocols.

Since the classical work of Keys and his associates over 40 years ago (35), there have been few systematic investigations of the behavioral consequences of food restriction under highly controlled conditions. It is apparent from the available literature that acute and chronic food restriction of the extent proposed here may have comparatively subtle behavioral effects on brain function and behavior. In this series of energy-deficient studies we sought to exploit the opportunity to assess changes in mental performance attributable to food restriction using modern behavioral methods.

## **Methods**

### Subjects

The subjects recruited for both, the short-term starvation study and the long-term energy restriction this study were young adult men and women between the ages of 18-30yr. Subjects were excluded from participating for the following reasons: (a) Subjects with any acute or chronic disease or who are using drugs that the physician and principal investigator decide would interfere with the normal adaptation to the proposed

intervention. (b) Subjects with any physical disability that might place them at risk during the dietary modifications and tracer experiments. (c) In order to be chosen for entry into the study ideally both the men and women would be expected to meet military body-weight and percent body-fat standards (36). However, because of the limited pool available for inclusion in our studies the majority of our subjects were drawn from the entire ethnic and racial spectrum available to us at MIT and within the Boston/Cambridge area. Recruitment procedures for those in the local community included advertisements in different living group areas at MIT. A serious attempt was made to include the mixture of minorities and racial backgrounds that are characteristic of the local student population. The minority representation within the MIT student population is 13% (with Asian accounting for 51%, Black 39% and Hispanic 10%).

Subjects received medical and nursing supervision throughout the entire study. The subjects were fully informed of the purpose, nature and design of the experiments and the potential hazards involved and they signed consent forms. They were allowed to continue with their normal everyday activities but were requested to maintain a relatively constant level of physical activity during the experimental period.

### Statistics

Data have been summarized as mean $\pm$ SD. Descriptive statistics were generated for each variable, by gender and study phase. For the longer-term study variables were analyzed using mixed models analysis of variance, with factors for gender, study phase and the gender by phase interaction; non-significant factors were removed from the model in a hierarchical fashion beginning with the interaction term. The models for leucine oxidation, glucose production and glycerol kinetics in which the infusion period is

divided up with respect to exercise included factors for gender, study phase. Relative exercise period, and all the interactions between factors; non-significant factors were removed from the model in an hierarchical fashion beginning with the 3-way interaction term. For all models, contrasts were used to make any pairwise comparisons of interest, as appropriate from the final model. All p values are two-sided and are not adjusted for multiple comparisons or tests. Data were analyzed using SAS version 8.00 (SAS Institute, Inc., Cary, NC).

### Experimental Designs

#### **Short-term, Complete Fasting Studies: (Fig. 1S)**

##### Approach

In this study subjects were fed for 6 days a standardized meat-free diet, providing about 45 kcal/kg/d, and 1.2 g/kg/d protein. The latter was provided via an egg-protein-based drink. Non-protein calories were divided as 40% fat and 60% carbohydrate. The diet was  $^{13}\text{C}$ -neutral, to allow a relatively steady background (natural)  $^{13}\text{C}$ -abundance in breath  $\text{CO}_2$ , in preparation for the tracer phase of the experiment, as described previously. Subjects ingested 3 meals per day, at 0800 h, 1200 h, and 1800 h, under supervision of investigators and/or dietary staff at the M.I.T. Clinical Research Center.

The fasting/metabolic studies were conducted at the CRC and M.I.T. Medical Department (under medical supervision). Between each of the different 3-hourly isotope intravenous infusions, sedentary activity was allowed. Water was allowed ad-libitum (but was to exceed 2 liters), and multi-vitamin-mineral tablets are given on a daily basis, as well as salt tablets ( $\text{NaCl}$ ) (about 4 g per day) and potassium supplements (K-

LYTE®) (about 40 meq per day). Vital signs were measured every 6h and gown weight was recorded. Blood glucose and electrolytes were monitored daily.

Twenty volunteers participated in this study: 10 males and 10 females. All subjects tolerated the 3d-starvation except one female volunteer who elected to terminate her participation in the study at the end of day 1.

The tracer studies (Fig. 2S) involved constant intravenous infusions of; (a) [ $^2\text{H}_5$ ]glycerol; 6.6  $\mu\text{mol/kg/h}$ , (b) [6,6, $^2\text{H}_2$ ]glucose: 13.2  $\mu\text{mol/kg/h}$ , (c) [1- $^{13}\text{C}$ ]leucine: 2.8  $\mu\text{mol/kg/h}$ , (d) [2,2- $^2\text{H}_2$ ]palmitate: 2.4  $\mu\text{mol/kg/h}$  (following binding to albumin).

Prior to each 3-hour infusion, baseline samples (blood and breath) were taken to assess background isotopic abundance in plasma molecules (glycerol, glucose, leucine, palmitate) and breath  $^{13}\text{CO}_2$ . Throughout each 3-hour infusion, breath samples and blood samples (8 ml) were taken for subsequent analyses. Indirect calorimetry, using a ventilated hood, was performed during the 2nd and 3rd hour of each infusion, to assess total  $\text{CO}_2$  production ( $\text{VCO}_2$ ), energy expenditure, and the utilization of fuels.

Plasma glycerol flux was used as a marker of whole-body lipolysis and glucose flux a measure of glucose production and uptake. The "leucine" technique allows measurement of leucine oxidation, protein oxidation, protein synthesis and protein breakdown. Palmitate flux was used as a marker of free fatty acid release and reesterification. Details of methods of analysis for this short-term, complete fast study are provided in the original cooperative agreement proposal and are not repeated here.

One female and one male volunteer received two 5h  $^{13}\text{C}$ -Na bicarbonate infusion on each starvation day. The purpose was to obtain bicarbonate recovery data to correct oxidation data. In addition, one female and one male did not receive any tracer, but

underwent the 3d starvation period in the CRC. Here breath samples were obtained as a sham to correct for substrate oxidation data.

### Major findings

Body composition data in all subjects is summarized in Table 1.

Total CO<sub>2</sub> production and O<sub>2</sub> consumption, <sup>13</sup>CO<sub>2</sub> and plasma enrichments were measured in all subjects and these data have been used to calculate: leucine oxidation, carbohydrate and lipid oxidation, plasma <sup>13</sup>C-KIC (leucine metabolite), N<sub>15,15</sub>, urea, <sup>2</sup>H<sub>2</sub> glucose, <sup>2</sup>H<sub>5</sub> glycerol and <sup>2</sup>H<sub>2</sub> palmitate kinetics. The mean kinetic parameters are summarized in Table 2.

The changes in the oxidation of leucine, carbohydrate and lipid during the 3-day fast showed a similar response in both men and women. Carbohydrate oxidation declined (Fig. 3S) as the fast continued while lipid and leucine oxidation increased (Fig. 3S). The decline in carbohydrate oxidation was associated with a decline in the rate of plasma glucose appearance (a measure of glucose release from the liver) (Fig. 4S). Similarly, the increased rate of whole body lipid oxidation was paralleled in both men and women by increased rates of glycerol and palmitate appearance (Fig. 4S). This would be expected where lipolysis with subsequent fatty acid transport to the liver and fatty acid oxidation is stimulated as a consequence of energy restriction over the 72h fast period.

The increased rate of leucine oxidation amounted to about a 30% rise between the initial overnight fast and the end of the 72h restriction period. This increase was similar in men and women with whole body leucine turnover (leucine Ra) (Fig. 5S)

showing an essentially comparable change over this period of time. Furthermore, the rate of urea appearance showed a tendency to rise between the end of the first and third days (Fig. 5S). This would be expected if body protein was contributing a higher proportion of the energy expenditure under these conditions of a fast (Fig. 6S). As also anticipated, total nitrogen excretion via the kidney was also higher at the end of the 72h period of fast. Again the response of men and women appeared to be quite similar.

### Summary and conclusions

These consecutive substrate kinetic studies were conducted to assess the effects of a 72h complete fast in healthy military-eligible men and women. The pattern of change in substrate utilization in response to the complete fast revealed an expected shift toward the oxidation of body fat with the contribution by carbohydrate decreasing. The pattern and quantitative changes were similar in men and women. Thus, it is concluded that a short-term period of complete caloric deprivation has a similar impact on the level and partitioning of energy substrates in healthy, military eligible men and women.

### **Longer-term Partial Caloric Restriction Studies (Fig. 1L)**

#### Approach

Twenty four subjects(11 males and 13 females) were enrolled for the study. Seven subjects were unable to do the infusion-related component of the study. The reasons included poor laboratory values, difficulty in obtaining blood samples during the infusion, difficulty with the extended time required for the protocol and other personal

reasons. Thirteen subjects (7 male and 6 female) completed the 41-day protocol. Three female subjects withdrew from the study, citing personal reasons. One male did not show sufficient compliance with the protocol and was terminated from the study on day 12 of the 41-day period.

### Study Duration and Phases

This study was carried out over a period of 41 days. It consisted of 3 phases.

Phase 1: weight-maintenance diet for 10 days.

Phase 2: 70% of previous caloric intake for a period of 3 weeks

Phase 3: ad libitum feeding and observation period lasting for 10 days.

### Design of 24 hour tracer infusion, substrate kinetic studies (Fig. 2L)

A series of three, 24-hour metabolic experiments was conducted (Phase, 1 day 10; Phase 2, days 17 and 31), each started at 1800h with a 12h feeding phase during the second half of the 24-hour experiment, to decrease the likelihood of recycling (into plasma) of tracers (Fig. 2L). The last meal preceding the 24-hour metabolic experiment was completed at 1600h with 10 isocaloric/ isonitrogenous meals being given during the infusion study at hourly intervals. The macronutrient content of the diet was identical to that given during the days preceding the 24-hour infusion, except that the daily intake was provided as small frequent meals and based on egg protein, lipid and carbohydrate (wheat starch) to match their previous intakes of protein and energy. Subjects rested (half-sitting or supine position) during most of the 24-hour experiment. They slept

between 0000h and 0600h. During the latter interval, blood  $^{13}\text{CO}_2$  was measured, as previously described.

A physiological-challenge involving an exercise period of 90 min took place from 2100 h to 2230 h (Fig. 3L). This involved having subjects cycle on a bicycle ergometer, at 55% of  $\text{VO}_2$  max (the latter was measured in each subject, prior to initiation of the dietary study).

Three-hourly urine collections were performed throughout the 24-hour experiment, for measurement of volume, total N, urea N, ammonia N, creatinine, 3-methyl-histidine by methods routinely used in our laboratories. Throughout the 24-hour tracer experiment a primed intravenous infusion of [ $^{15}\text{N}$ ,  $^{15}\text{N}$ ]urea (7mmol/kg/h) was given to measure total urea production (net protein oxidation) from plasma urea flux. Simultaneously, a 24-hour primed intravenous infusion of [ $1\text{-}^{13}\text{C}$ ]leucine (2.8 mmol/kg/h) was performed to assess whole-body leucine (and protein) oxidation.

In addition, prior to, during and following the 90 min exercise challenge (2100 h – 2230 h) an additional 5-hour tracer protocol was conducted to evaluate the subject's metabolic response to moderate physical activity. Here primed constant infusions of [ $6,6\text{-}^2\text{H}_2$ ]glucose (0.22 mmol/kg/min; prime 17.6 mmol/kg, [ $^2\text{H}_5$ ]glycerol (0.1 mmol/kg/min, prime 1.5 mmol/kg), [ $2,2\text{-}^2\text{H}_2$ ]palmitate (0.04 mmol/kg/min, no prime) were given for two hours prior to the exercise phase.

Subjects then started the 90-min exercise period and immediately following this they rested in bed, with the tracer infusions continuing for a further 90 min. Immediately after the start of exercise the glycerol infusion rate was changed, following an exponential pattern, to achieve an infusion rate 1.5 times the resting infusion rate. The

glucose infusion rate was also increased to 2 times the initial rate. These changes were achieved within 15-20 mins. During recovery the infusion rates were decreased following the same pattern. Our purpose of changing the infusion rate was to minimize changes in the isotopic enrichment of the plasma pools during the non-steady state in the transition from rest to exercise and return. The volume of infusate given during the 24-hour experiment was within the range of 8–10 ml/hour when glucose, glycerol and palmitate infusate was added, the volume infused was 15–20 ml/hour.

#### Blood sampling

Blood sampling was performed at intervals throughout the 24-hour experiment after sampling 2 baselines (-15 and -10 min, prior to time 0). During the last 60 min of exercise and first 30 min of recovery blood (8 ml) was drawn every 10 min. Sampling was every 20 min during the last hour of recovery.

#### Breath sampling for $^{13}\text{CO}_2$ analysis

In parallel to blood samples, breath samples were drawn for  $^{13}\text{CO}_2$  analysis. Breath gas were collected in disposable rubber bags by a mechanism that permits removal of dead-space air. The air sample was then transferred into two, 15-ml non silicon-coated glass tubes (Venoject, Terumo Medical Corp, Elkton, MD). Samples were stored at room temperature until analyzed by isotope ratio mass spectrometry (MAT Delta E; Finnigan, Bremen, Germany).

### Wingate Test

Maximum anaerobic power and capacity was assessed over a 30 s interval (Wingate test) in the normocaloric and hypocaloric phases of the study. Subjects' feet were firmly strapped onto the pedals of the bicycle, seat height was adjusted and subjects allowed to pedal against the natural resistance of the ergometer for 20 s., following which resistance was pre-loaded onto a weight pan at 0.75 kg/kg body weight. Power output, peak power output, total work (average 30 s output) and fatigue index were calculated for each session.

### Dual energy x-ray absorptiometry.

Bone mass and soft tissue composition were measured using dual energy x-ray absorptiometry (DPX-Plus, Lunar Corp., Madison, WI), using methods described by Mazess et al (37) and Friedel et al (38,39). Absorptiometry measurements were made at the time of subject selection, and at the end of Phases 1 and 2 of the hypocaloric studies. Subjects were measured using a 30-minute Scan, and each time with the same instrument the subject was originally measured on at the initiation of the study.

### Strength and endurance performance tests

Maximal dynamic lift capacity was measured at the end of Phases 1 and 2 using instrumentation and methods according to those described previously (40). For this procedure, weights are added to a carriage, to which handles are attached, and the carriage slides upward on a vertical track. The subject begins lifting 18 kg, followed by the addition of weight in each subsequent trial in increments of 18 kg until difficulty is

encountered. Additional weight was added in increments of 9 kg until a maximum lift ability is achieved. A successful lift was defined as one in which the subject is able to raise the weighted carriage to shoulder height in one clean motion.

#### Vertical jump power

This test was used in Range II and showed decrements. It was easily performed and is a “real life” assessment of muscle performance. The procedure and method of assessment was followed as described by Harman et al (41).

#### Tests of mental performance

The following tests were performed : computer-based tests of performance and mood that: 1) have previously been found to be sensitive to nutritional manipulations 2) assess whether certain critical behavioral functions that are essential for military performance are altered by acute or chronic food restriction per se. The battery of mental performance tests is fully automated and requires approximately one hour to administer, using IBM-compatible laptop computers.

The tests were administered twice a week; before lunch during the prolonged hypocaloric studies (Series II). Prior to food restriction subjects had sufficient practice on each task to ensure they had reached a stable level of performance. To assess mood the Profile of Mood States (POMS) (42,43) was used.

### Activity

Motionlogger Actigraphs, Model AMA-32 (Precision Control Devices, Ft. Walton Beach, FL) were employed to continuously assess patterns of rest and activity during the study. All subjects were monitored during the entire period of study, as well as the baseline pre-admission period. The devices were worn on the wrist of the non-preferred hand using a standard wristwatch band. Each device contained a microcomputer, 32k of memory, an analog-to-digital (AD) converter and a piezoelectric sensor. It is powered by standard wristwatch batteries. Data collected by the AMA-32 was downloaded to an IBM-compatible computer via an interface device for analysis using the ACTION computer program (Ambulatory Monitoring, Inc., Ardsley, NY). Our collaborators had previously employed such devices to assess diurnal patterns of rest and activity in a variety of populations.

### Wilkinson Auditory Vigilance Task

The Wilkinson auditory vigilance task (44) is a measure of sustained auditory vigilance. Every 2 seconds, for a 30-minute time period, a 400 ms tone is presented via headphones. To mask extraneous stimuli, white noise was also presented. Forty of the tones were approximately 70 ms shorter than the rest. The subject must correctly identify these signal tones by pressing a key on the computer when he/she believes a signal tone has been presented) were recorded. Unlike the original version of this test, the difficulty is equalized for each subject by varying the duration of the test stimuli. This was accomplished during the practice session when each subject's performance was

set at approximately 50% correct. Once a test stimuli was determined for a subject it was held constant across all experimental sessions.

#### Four-Choice Reaction Time Task

The four-choice visual reaction resembles the Wilkinson four-choice reaction time task and measure visual vigilance (44). The subjects were presented with a series of visual stimuli at one of four locations on a computer monitor and would indicate, by striking one of four adjacent keys on the microcomputer keyboard, the correct location of each stimulus. Five hundred trials were administered. In addition to response latency for each trial, errors of omission (response latency greater than 1 second) and commission were recorded. The task duration was about 10 minutes.

#### Calculation Performance Task

This test, modeled after one described by Klein et al (45), presents the subject with a series of 125 randomly generated pairs of 2-digit numbers. The subject's task was to sum as many pairs as possible in the allotted four-minute time interval. The test scored according to a number of calculations completed in the time allowed, irrespective of accuracy. The duration of the task was approximately 4 minutes.

#### Tracking Task

This was a two-dimensional compensatory task in which a small cursor continuously moved in random directions on the display. The subject used a trackball to control the cursor and compensate for the directional changes in its movement in order

to keep it positioned at the center of a large set of crosshairs on the screen. Analyses were based on the RMS error of the cursor position relative to center of the crosshairs (i.e. the time-averaged Euclidean distance from the crosshairs' center). The duration of the task was approximately 5 minutes. The tracking task measured psychomotor performance similar to that required in aircraft piloting.

#### Profile of Moods States (POMS)

The POMS consisted of 65 adjectives each rated on a 5- point scale. When analyzed it yielded 6 factors: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia and Confusion-Bewilderment (42,46).

#### **Sample Analysis**

##### Isotopic and non-isotopic analyses in plasma

The  $^{13}\text{C}$  abundance in ketoisocaproate (KIC) (for  $^{13}\text{C}$  leucine) was determined after derivatization using quinoxalinol-t-BDMS, using gas chromatography/mass spectrometry and selective ion monitoring (SIM), as previously described (47). Plasma [di- $^{15}\text{N}$ ]urea enrichment also was determined as previously described (47) using the t-butyltrimethyl-silyl derivative. Plasma glucose concentration was measured using a glucose analyzer (Beckman Instruments) and the glucose oxidase method. The measurement of enrichment of [6,6- $^2\text{H}_2$ ]glucose in plasma has been previously described (48). Isotope enrichment was determined using a pentaacetate derivative by gas-chromatography/mass-spectrometry (GC/MS), with electronic impact ionization and selective ion monitoring at the mass-to-charge ratios (m/e) 202, 201 and 200. FFA was extracted from plasma, isolated by thin layer chromatography and derivatized to form

their methyl esters. Palmitate and total FFA concentrations were determined by gas-chromatography using heptadecanoic acid as internal standard. Isotopic enrichment of palmitate was measured by BC/MS analysis of the methyl ester derivatives. Ions at m/e 270 and 271 were selectively monitored (49). Isotopic enrichment of glycerol was determined as described previously (49,50). Glycerol concentrations was measured by enzymatic and calorimetric assay (Technicon RA-500, Tarrytown, NY).

Amino acid levels in plasma and infusates were determined using HPLC. Infusates of the tracers were analyzed in duplicate. Plasma and urinary nitrogen concentrations were determined by means of a modified version of the procedure of Marsh et al (51) with an autoanalyzer. Urinary urea excretion was corrected for changes in the body urea pool (as previously described (47)). Total urinary nitrogen concentrations were determined by microKjeldahl analysis.

### **Evaluation of primary kinetic data**

Leucine oxidation, turnover and by extrapolation to whole body protein metabolism were computed, as previously described (47). The approach was based on the two-pool whole-body model of leucine metabolism described originally by Matthews et al (52 ).

Urea production was computed, as previously described (47) from [<sup>15</sup>N<sup>15</sup>N]urea enrichment data. Glucose, glycerol and palmitate fluxes also were computed as we have described (21) and estimates for adipose tissue lipolysis and free-fatty acid reesterification were made.

Energy expenditure was calculated from indirect calorimetry using the Weir equation (53) and utilization of substrates for energy was estimated using equations by Garlick (54).

## **Results and discussion of the longer-term partial energy restriction in men and women**

Thirteen subjects (7 male and 6 female) completed the study. The general characteristics of subjects that participated in this study are described in Table 1L. For some but not all subjects, it was possible to study them at an intermediate period of the hypocaloric phase and these data are also presented. Statistical analysis was carried out to elicit differences between the genders as well as the effect of phases (normocaloric and hypocaloric) on measured parameters. All phase-dependent statistical differences reported for metabolic parameters refer to the infusion studies carried out during the normocaloric phase and the second infusion during the hypocaloric phase.

### **Body composition**

The body compositional characteristics of male and female subjects at the end of the normo-caloric and hypocaloric phases of the experiment are summarized in Table 2L. Relatively small but statistically significant decreases in body weight, %body fat and lean tissue were observed in both genders. Body weight decreased from  $72.2 \pm 11.7$  Kg to  $70.8 \pm 10.5$  Kg in male subjects and from  $59.1 \pm 4.9$  to  $57.7 \pm 4.8$  Kg in female subjects. Body fat decreased from  $15.2 \pm 3.3$  to  $14.5 \pm 3.7$  % in males and from  $29.3 \pm$

7.4 to  $28.0 \pm 8.5$  % in female subjects. A small but significant difference was observed with BMC decreasing from  $3.6 \pm 0.7$  Kg to  $3.6 \pm 0.7$  Kg in males and from  $2.7 \pm 0.5$  to  $2.7 \pm 0.5$  Kg in females. Lean mass decreased from  $61.3 \pm 11.0$  to  $60.7 \pm 10.5$  Kg in males and from  $41.8 \pm 5.7$  to  $41.5 \pm 5.9$  Kg in female subjects.

#### Dietary characteristics

The dietary intakes during the three phases (normocaloric, hypocaloric and adlib) are summarized in Table 3L. The hypocaloric phase was created by a mean reduction of ~ 1000 Kcals/day (~30% of usual caloric intake) in both men and women. The total protein intake was maintained at levels that, for subjects who are in energy balance, would be more than sufficient to maintain body nitrogen homeostasis. The reduction in energy intake was achieved by approximate isoenergetic restrictions in the carbohydrate and lipid components of the diet.

Energy intake during the 10-day ad libitum phase was higher than during the normocaloric phase. This might suggest a compensatory adaptation to energy intake following a period of energy restriction or possibly due to a higher rate of energy expenditure. We have observed previously such a compensatory increase in energy intake after a restriction period in healthy adult men (55).

## **Metabolic parameters**

### Leucine Oxidation

Leucine oxidation was used as a marker of the consequences of the partial energy restriction on whole body amino acid status. The results are summarized in Table 4L.

### 24 h Leucine Oxidation

The 24h estimates of leucine oxidation were arrived at by two means; (1) summation of all phases of the study, including exercise and recovery and (2) by extrapolation of the post-recovery component of the study. No significant differences in 24h-leucine oxidation, calculated by both these methods was observed between dietary periods as well as between genders (Figs. 4L and 5L). Hence, on a daily basis there appears to have been an adaptive, protein (or leucine) sparing response to the energy restriction and that this was equally effective in the two genders.

### Leucine Oxidation during Exercise

Exercise, as anticipated, resulted in a profound increase in leucine oxidation during the 90 minute period and a decrease during the following 90 minute recovery period. No statistical differences were elicited between the genders during the normocaloric phase. However, female subjects, when compared to males, demonstrated significantly lower oxidation values during the hypocaloric phase. Thus, it appears that females do not utilize as much branched-chain amino acid during exercise as do males under conditions of a partial energy restriction.

When an analysis was carried out based on phase, in female subjects, leucine oxidation was significantly higher during the normocaloric phase (as compared to the hypocaloric phase). This pattern was also observed in female subjects during the recovery phase. No such differences were elicited in male subjects. Thus, it appears that during and immediately after exercise leucine oxidation is induced less when they are adapted to a hypocaloric diet. However, there must be compensations during the remainder of the 24h period such that the response of daily leucine oxidation is similar for both males and females within this pattern of exercise and range of energy intake.

#### Plasma leucine concentrations

The 24 h pattern of plasma leucine concentrations for each phase of the study is shown in Fig. 6L. There were no differences between genders and so the data have been combined. There was no consistent effect of energy intake on the plasma leucine response or concentrations.

#### Glucose production

Deuterated glucose was used to estimate glucose production and results are summarized in Table 5L.

#### Total glucose production (baseline, exercise and recovery)

For the mean value of glucose production over the infusion (baseline, exercise and recovery) period, no differences were elicited between the genders. However, mean values were significantly higher during the normocaloric phase of the study in both genders (Figs. 7L and 8L).

### Glucose production during exercise

Glucose production during exercise and recovery phases in both genders was significantly higher in the normocaloric phase of the study. This pattern was also observed during the recovery phase. In the smaller subset of subjects, an intermediate study was carried out during the hypocaloric phase. Data from the intermediate hypocaloric phase also illustrates a lower rate of glucose production when compared to the normocaloric phase.

These findings are consistent with an expected reduced rate of glucose appearance when energy intake is subnormal. However, it further appears that there are no differences in the response of males and females under these defined, experimental conditions.

### Glycerol kinetics

Rate of appearance (Ra) of Glycerol, an index of lipolysis was studied using labeled glycerol. Results are tabulated in Table 6L.

### Total glycerol Ra (baseline, exercise and recovery)

There were no differences between genders for the mean Ra (rate of appearance) of glycerol through the infusion study. However, Ra of glycerol during the normocaloric phase was significantly lower than during the hypocaloric phase in both genders. In male subjects, the Ra of glycerol increased from  $3.09 \pm 0.68$   $\mu\text{mol/kg/min}$  in the normocaloric phase to  $3.94 \pm 0.60$   $\mu\text{mol/kg/min}$  in the hypocaloric phase. A similar pattern was observed in female subjects wherein values increased from  $3.14 \pm 0.41$  to  $3.56 \pm 0.30$   $\mu\text{mol/kg/min}$ , for the normocaloric and hypocaloric phases respectively.

### Glycerol Ra during exercise

During the exercise phase, no differences between the genders was observed for both, the normocaloric as well as the hypocaloric phase. Further, no differences between the exercise phase and recovery phase, expressed per kg per min were observed. However, during exercise, the Ra of glycerol was significantly lower during the normocaloric phase as compared to the hypocaloric phase.

Again, it would appear that the mobilization of lipid occurs similarly in men and women when the energy intake is moderately hypocaloric.

### Palmitate kinetics

Palmitate kinetics was measured using  $^{13}\text{C}$ -palmitate. Data for the study period is presented in Table 7L. Palmitate kinetics demonstrated the same pattern as glycerol with lower values observed in both genders in the normocaloric phase. An increase in the Ra, palmitate was observed through all phases of the study during the intermediate phase of study of the hypocaloric phase. This increase was even more marked in the latter part of the hypocaloric phase

### Urea production

Rates of Urea production are summarized in Table 8L. The 24h rates are given without reference to shorter periods because the size and relatively slow rate of whole-body urea turnover. There was no effect of gender observed through the study. However, the rate of urea production during the normocaloric phase was significantly lower in both, male and female subject as compared to the hypocaloric phase, indicating

a reduced efficiency of protein utilization during this latter phase. As anticipated, there was a diurnal rhythm to urea production with a decline in the urea appearance rate during the fed period of the day (Fig. 9L and 10L).

#### Total energy expenditure

Total energy expenditure was measured using doubly labeled water while subjects continued their daily activities during the normocaloric phase (Day 1 of the 41-day study period) and during the hypocaloric phase (Day 21-28 of the 41 day study). There was a significant decrease in the TEE (table 9L) during the hypocaloric phase in both genders, with the decrease being larger in male subjects. This decrease was also observed when TEE was calculated from the calorimetric data during the infusion studies. The calorimetry derived values were obtained during the infusion study which included a period of exercise as well as restriction to bed. However, the doubly-labeled water estimates would imply that there was a voluntary reduction in physical activity as a consequence of the hypocaloric intake. This had not been fully anticipated and we encouraged the subjects to continue with their usual everyday activities.

The values obtained by the doubly labeled water and calorimetric methods reflected the decrease in caloric intake during the hypocaloric phase. There were no significant differences in the Respiratory quotient (RQ) and Food Quotient (FQ) between the phases in both genders.

### Physical Performance

Measures of physical activity were obtained in several ways, including the Wingate Test and Maximum Dynamic Lift. For peak power, there were no differences in female subjects between the two phases, while peak power in males, during the hypocaloric phase was significantly lower.

Vertical jump was significantly lower between the two phases in both genders. All other parameters did not demonstrate any differences.

### Mental performance

Regular behavioral testing and activity monitoring were conducted on the subjects in the chronic under-nutrition protocol. A total of 11 behavioral test sessions and one practice test session were administered throughout all phases of the study. Complete or near complete data sets were collected on all test subjects. Four cognitive tests were administered on laptop computers: Four-choice visual reaction time, The Visual vigilance test, the Mental mathematics test and the psychomotor tracking task. In addition, the Profile of Mood States was administered at every behavioral test session - this standardized test provides information on six cognitive states: vigor, fatigue, confusion, anxiety, anger and depression. Data reduction and analysis for this very large data set are currently underway. In addition, data on patterns of rest and activity using wrist actigraphs (AMA-32; Precision Control Devices) were collected during each phase of the study. Due to equipment malfunction and poor subject compliance a full data set for every subject was not obtained and sufficient activity data for statistical analysis may not be available. Descriptive data on multiple subjects will, however, be

available once preliminary data reduction and analysis of the activity data are completed by our colleagues at USARIEM.

The behavioral and activity data from this study are a unique and massive set that may provide important new information on the effects of chronic under-nutrition on healthy human (female and male) cognitive status, mood and patterns of rest and activity.

### **Summary overview of the two investigations**

These two investigations, one involving a short-term (72h) complete fast and the other a 3-week hypocaloric intake (amounting to about a 30% reduction from a eucaloric level) were conducted to determine whether there are differences in the whole-body metabolic responses between men and women of military age and eligibility. Substrate kinetics related to amino acid, lipid and glucose metabolism were measured, together with total energy expenditure and supplemented with physical and mental performance tests. The hypocaloric experiment included an assessment of a 90 min exercise period (55%  $\text{VO}_2\text{max}$ ) conducted in the evening between 2100h and 2300h, and six hours after the last meal.

The metabolic response to total the 72h starvation showed the characteristic changes in energy substrate metabolism (increased dependence on lipid, reduced utilization of glucose). They were not different between men and women.

The response to the long-term hypocaloric diet was also consistent with an expected small loss of body weight and small increases in lipolysis and decrease in

glucose production. The changes were not profound nor were they different, in the aggregate between men and women.

A number of observations should be made in reference to this study; first, these data are relatively unique in that we studied the effects of significant but not severe energy restriction. Most studies of this kind have involved more profound and/or very low energy diets which have involved also reductions in total macronutrient intake, including protein. Here the profile of macronutrient intake was maintained. Second, our subjects were studied under highly controlled and relatively constant environmental conditions. The impact of additional stress, perhaps that created by continued and marked exertion would have elected possible difference in gender response, as we had hypothesized at the outset. Nevertheless, these highly controlled studies it is apparent that men and women respond similarly to an acute, 3-day fast and a more prolonged period at a sub-optimal energy intake.

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**Table 1L. Characteristics of subjects who participated in the long-term investigation of partial energy restriction**

	<b>Males</b>	<b>Females</b>
Age (years)	21.6 ± 1.8	22.1 ± 2.1
Height (cms)	178.5 ± 6.4	162.3 ± 3.4
Weight (kg)	58.9 ± 3.7	82.4 ± 2.8

Values are Mean±SD

**Table 2L. Body composition of subjects who participated in the long-term investigation of partial energy restriction**

	Males		Females	
	Normocaloric Phase (NC)	Hypocaloric Phase(HC-2)	Normocaloric Phase (NC)	Hypocaloric Phase (HC-2)
Body Wt. (Kg)	72.2 ± 11.7	70.8 ± 10.5*	59.1 ± 4.9	57.7 ± 4.8*
Fat (%)	15.2 ± 3.3	14.5 ± 3.7*	29.3 ± 7.4	28.0 ± 8.5*
Bone Mineral Content (g)	3629.2 ± 738.7	3613.1 ± 740.1*	2710.0 ± 532.7	2682.6 ± 294.9*
Lean tissue (Kg)	61.3 ± 11.0	60.7 ± 10.5*	41.8 ± 5.7	41.5 ± 5.0*

Values are mean ± SD

\*p<0.05, effect of phase, gender independent.

**Table 3L. Dietary intakes of subjects who participated in the long-term investigation of partial restriction**

	Males			Females		
	Normocaloric phase	Hypocaloric phase	Adlib phase	Normocaloric phase	Hypocaloric phase	Adlib phase
Energy(kCal/d)	3688 ± 332	2560 ± 229	4056 ± 1661*	3131 ± 486	2180 ± 357	2878 ± 716
Protein (g/d)	85.4 ± 11.3	83.3 ± 11.1	174.6 ± 60.1*, **	72.1 ± 7.9	70.1 ± 7.9	90.4 ± 16.5
Carbohydrate (g/d)	500.2 ± 42.3	345.3 ± 28.3	505.9 ± 219.8*	425.9 ± 71.1	295.5 ± 54.4	443 ± 135*
Fat (g/d)	149.6 ± 14.0	93.9 ± 8.9	148.3 ± 62.0*	126.6 ± 20.9	79.8 ± 14.1	82.6 ± 22.7**
Protein (%)	9.2 ± 0.7	13.0 ± 0.9	17.5 ± 1.9*, **	9.3 ± 1.1	13.0 ± 1.7	12.9 ± 2.3**
Carbohydrate (%)	54.3 ± 0.7	54.0 ± 1.0	49.7 ± 1.7*, **	54.3 ± 0.8	54.1 ± 1.3	61.0 ± 4.9*, **
Fat (%)	36.5 ± 0.4	33.0 ± 0.5	32.8 ± 2.1**	36.3 ± 0.4	32.9 ± 0.4	26.1 ± 5.5**

Values are mean ± SD

\* p<0.05, Adlib phase vs hypocaloric phase

\*\* p<0.05. Adlib phase vs normocaloric phase

Table 4L. Leucine oxidation during the different phases of feeding

	Males			Females		
	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Hypocaloric phase (HC-2)	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Hypocaloric phase (HC-2)
<b>24-hr</b>						
μmol/kg/d	1174.5±385.3	788.5±164.9	1004.9±258.7	1307.8±536.9	1182.7±10.5	1140.2±329.2
mg/kg/d	153.9±50.5	103.3±21.6	131.6±33.9	171.3±70.3	154.9±1.4	149.4±43.1
<b>During exercise</b>						
μmol/kg/30 min	68.1±38.0	29.1±28.9	35.1±26.2	79.2±51.4	54.4±22.2	61.2±34.9*
<b>Recovery</b>						
μmol/kg/30 min	13.1±3.7	8.1±2.2	11.0±4.2	16.9±9.4	7.6±0.2	8.2±5.7*
<b>Post exercise</b>						
μmol/kg/d (extrapolated to 24h)	632.6±146.4	576.3±24.6	728.5±115.0	673.0±143.2	844.9±178.9	698.8±91.6

Values are mean ± SD

No differences between phases for both genders for 24 h leucine oxidation

\*p<0.05, females during exercise, recovery between phases

**Table 5L. Glucose production of subjects who participated in the long-term investigation of partial energy restriction**

	Males			Females		
	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Hypocaloric phase (HC-2)	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Hypocaloric phase (HC-2)
Baseline	4.55 ± 0.98	3.56 ± 0.95	3.35 ± 0.78	4.01 ± 1.34	2.69 ± 0.44	2.94 ± 0.53
Exercise	3.61 ± 0.60	2.89 ± 0.39	2.95 ± 0.27 *	3.12 ± 0.71	2.29 ± 0.31	2.70 ± 0.65 *
Recovery	3.38 ± 0.46	2.55 ± 0.46	2.62 ± 0.32 *	2.92 ± 0.63	2.23 ± 0.14	2.59 ± 0.49 *
Mean (throughout study)	3.65 ± 0.49	2.84 ± 0.38	2.87 ± 0.26 *	3.16 ± 0.75	2.32 ± 0.25	2.69 ± 0.55 *

Values are mean ± SD (mg/kg/min)

Mean (throughout study): average through study (including baseline, exercise and recovery phases)

\*p<0.05; differences between phases NC and HC-2, both genders

**Table 6L. Glycerol kinetics of subjects who participated in the long-term investigation of partial energy restriction**

$\mu\text{mol/ Kg/min}$	Males			Females		
	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Hypocaloric phase (HC-2)	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Hypocaloric phase (HC-2)
Baseline	4.20 $\pm$ 1.34	3.89 $\pm$ 1.12	4.69 $\pm$ 2.31	3.20 $\pm$ 0.91	3.77 $\pm$ 1.36	3.94 $\pm$ 1.09
Exercise	3.05 $\pm$ 0.87	3.79 $\pm$ 0.78	3.76 $\pm$ 0.76 *	2.96 $\pm$ 0.56	3.66 $\pm$ 0.27	3.69 $\pm$ 0.36 *
Recovery	3.14 $\pm$ 0.55	4.37 $\pm$ 1.79	3.71 $\pm$ 0.75	3.22 $\pm$ 0.45	3.13 $\pm$ 0.20	3.43 $\pm$ 0.54
Mean (throughout study)	3.09 $\pm$ 0.68	3.95 $\pm$ 0.94	3.74 $\pm$ 0.60 *	3.14 $\pm$ 0.41	3.40 $\pm$ 0.20	3.56 $\pm$ 0.30 *

Values are mean  $\pm$  SD ( $\mu\text{mol/kg/min}$ ).

Mean (throughout study): average through study (including baseline, exercise and recovery phases)

\* $p < 0.05$ ; differences between phases NC and HC-2, both genders

**Table 7L. Palmitate kinetics of subjects who participated in the long term investigation of partial energy restriction**

$\mu\text{mol/ Kg/min}$	Males		Females			
	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Hypocaloric phase (HC-2)	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Hypocaloric phase (HC-2)
Baseline	2.0±0.6	1.9±0.7	2.2±0.9	2.3±0.9	2.5±0.3	2.8±0.6
Exercise	2.6±0.9	3.2±1.4	3.6±1.1*	3.6±1.3	4.4±0.6	5.5±0.6*
Recovery	1.8±0.2	2.5±0.9	3.4±1.2*	2.6±0.5	3.2±0.4	4.0±1.3*
Total	2.2±0.5	2.8±1.1	3.6±1.1*	3.1±0.8	3.8±0.5	4.8±0.7*

Values are mean ± SD ( $\mu\text{mol/kg/min}$ ).

Mean (throughout study): average of exercise and recovery phases, baseline not included

\*p<0.05; differences between phases NC and HC-2, both genders

**Table 8L. Urea kinetics of subjects who participated in the long-term investigation of partial energy restriction**

	Males		Females	
	Normocaloric phase (NC)	Hypocaloric phase (HC-1)	Normocaloric phase (NC)	Hypocaloric phase (HC-1)
				Hypocaloric phase (HC-2)
μmol/kg/30min	2.04±0.18	2.71±0.45	2.09±0.22	2.34±0.24
				2.20±0.25*

Values are mean ± SD (μmol/kg/min)

\*p<0.05; differences between phases NC and HC-2, both genders

**Table 9L. Energy expenditure measured by doubly-labeled kinetics of subjects who participated in the long-term investigation of partial energy restriction**

	Males		Females	
	Normocaloric phase (NC)	Hypocaloric phase (HC-2)	Normocaloric phase (NC)	Hypocaloric phase (HC-2)
TEE (MJ/d)	14.97 ± 1.99	10.99 ± 1.27 *	12.86 ± 0.76	9.19 ± 0.94 *
TEE (MJ/d) (calorimetry)	14.03 ± 1.86	10.54±1.05 *	13.15 ± 2.04	8.64 ± 1.24 *
TEE (MJ/d) (Caloric Intake)	15.32 ± 1.16	10.75 ± 0.96 *	13.15 ± 2.04	9.16 ± 1.5 *
RQ	0.84 ± 0.02	0.86 ± 0.03	0.82 ± 0.03	0.83 ± 0.03
FQ	0.85 ± 0.03	0.84 ± 0.02	0.82 ± 0.01	0.82 ± 0.03

Values are mean ± SD

\* p<0.05; differences between phases NC and HC-2, both genders

\*\*P<0.05; differences between genders

**Table 10L. Physical performance of subjects who participated in the long-term investigation of partial energy restriction**

	Males		Females	
	Normocaloric phase (NC)	Hypocaloric phase (HC-2)	Normocaloric phase (NC)	Hypocaloric phase (HC-2)
Standing Reach Height	93.2 ± 6.2	93.2 ± 5.8	85.6 ± 4.0	85.1 ± 4.5
Vertical Jump Height (cm)	53.9 ± 10.1	50.6 ± 9.1*	31.6 ± 11.5	28.0 ± 8.7*
Max Dynamic Lift				
Maximum Lift Achieved (W)	147.5 ± 47.9	164.0 ± 42.9	78.0 ± 14.4	78.8 ± 20.6
Peak Power (W)	767.5 ± 244.8	840.2 ± 207.7*	521.8 ± 76.5	531.0 ± 113.9
Power/ kg BM (W/kg)	10.1 ± 2.2	10.7 ± 1.6*	8.3 ± 1.1	8.6 ± 1.5
Power/ kg FFM (W/kg FFM)	11.7 ± 2.0	12.4 ± 1.4*	11.5 ± 1.3	11.3 ± 1.5
Mean Power	672.3 ± 204.2	719.2 ± 188.4	435.6 ± 99.7	448.3 ± 91.9
Power/ kg BM	8.7 ± 1.5	9.1 ± 1.5	6.9 ± 1.4	7.3 ± 1.1
Power/ kg FFM	10.2 ± 1.5	10.6 ± 1.3	9.6 ± 1.7	9.5 ± 1.2
Fatigue Index	39.2 ± 10.8	40.4 ± 8.6	38.2 ± 16.3	40.5 ± 6.2

Values are mean ± SD

\* p<0.05; differences between phases NC and HC-2

**Table 1S. Body composition of subjects in the complete fast study**

	Males	Females
Body weight (kg)	78.7±8.7 <sup>1</sup>	58.9±8.6 <sup>1</sup>
Fat mass (%)	13.6±6.2	26.2±6.0
Fat Free Mass (%)	86.4±6.2	73.9±6.9

<sup>1</sup>Mean ±SD (n = 10).

Table 2S

Hours of Starvation

		12	24	36	48	60	72
Total Leucine Oxidation $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 16.5 $\pm$ 4.6	15.4 $\pm$ 3.1	21.1 $\pm$ 8.2	20.8 $\pm$ 5.4	22.5 $\pm$ 7.5	20.4 $\pm$ 5.6
		female 14.3 $\pm$ 1.9	11.2 $\pm$ 0.6	15.7 $\pm$ 3.8	19.5 $\pm$ 7.1	19.4 $\pm$ 3.3	18.8 $\pm$ 115.8
Total Carbohydrate Oxidation $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 6.3 $\pm$ 4.0	3.8 $\pm$ 2.2	4.7 $\pm$ 4.0	1.2 $\pm$ 1.3	2.8 $\pm$ 3.4	1.0 $\pm$ 1.3
		female 6.6 $\pm$ 3.1	3.6 $\pm$ 0.8	4.0 $\pm$ 1.8	2.6 $\pm$ 2.0	2.5 $\pm$ 2.4	1.7 $\pm$ 1.3
Total Lipid Oxidation $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 3.7 $\pm$ 1.3	4.4 $\pm$ 0.8	4.6 $\pm$ 1.5	5.9 $\pm$ 1.0	5.1 $\pm$ 1.2	6.0 $\pm$ 1.2
		female 3.6 $\pm$ 1.3	4.7 $\pm$ 0.8	4.7 $\pm$ 1.1	5.4 $\pm$ 0.7	5.3 $\pm$ 0.5	5.7 $\pm$ 0.7
Total Leucine Ra $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 100.3 $\pm$ 15.4	107.0 $\pm$ 14.0	113.6 $\pm$ 12.4	124.8 $\pm$ 19.3	118.9 $\pm$ 19.0	124.2 $\pm$ 16.9
		female 104.6 $\pm$ 20.9	105.9 $\pm$ 13.4	110.1 $\pm$ 8.7	120.5 $\pm$ 13.5	112.5 $\pm$ 15.7	121.6 $\pm$ 17.2
Total Leucine Ra (KIC) $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 114.6 $\pm$ 22.7	118.7 $\pm$ 19.8	123.1 $\pm$ 18.1	134.6 $\pm$ 16.6	135.1 $\pm$ 23.5	129.8 $\pm$ 28.9
		female 100.7 $\pm$ 10.8	90.6 $\pm$ 13.6	105.2 $\pm$ 14.4	105.9 $\pm$ 21.5	101.8 $\pm$ 18.9	107.0 $\pm$ 25.1
Total Glucose Ra $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 15.1 $\pm$ 4.3	12.6 $\pm$ 3.5	11.3 $\pm$ 3.1	11.0 $\pm$ 3.3	10.9 $\pm$ 2.6	10.4 $\pm$ 2.6
		female 13.4 $\pm$ 2.0	11.6 $\pm$ 1.5	9.8 $\pm$ 1.2	8.9 $\pm$ 0.9	8.8 $\pm$ 1.6	8.9 $\pm$ 1.0
Total Glycerol Ra $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 3.4 $\pm$ 0.9	4.7 $\pm$ 1.6	6.4 $\pm$ 3.0	6.7 $\pm$ 2.1	6.5 $\pm$ 2.0	6.3 $\pm$ 2.3
		female 4.1 $\pm$ 1.5	6.2 $\pm$ 2.5	8.0 $\pm$ 2.9	7.8 $\pm$ 2.3	7.6 $\pm$ 1.5	8.3 $\pm$ 4.0
Total Palmitate Ra $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 2.2 $\pm$ 0.7	2.9 $\pm$ 0.5	3.8 $\pm$ 1.3	4.3 $\pm$ 0.5	4.6 $\pm$ 2.1	3.5 $\pm$ 0.7
		female 2.1 $\pm$ 0.3	3.5 $\pm$ 0.8	4.6 $\pm$ 0.8	4.4 $\pm$ 0.8	4.6 $\pm$ 0.9	4.2 $\pm$ 0.6
Urea Ra $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$		male 372.4 $\pm$ 38.9	287.7 $\pm$ 22.4	394.4 $\pm$ 25.3	608.8 $\pm$ 11.8	367.1 $\pm$ 47.8	472.8 $\pm$ 35.5
		female 259.6 $\pm$ 26.8	221.1 $\pm$ 36.4	308.9 $\pm$ 47.8	476.0 $\pm$ 69.1	275.8 $\pm$ 23.0	363.3 $\pm$ 11.2
Total Urinary Nitrogen gr/12h		male 7.0 $\pm$ 1.9	6.9 $\pm$ 2.1	6.0 $\pm$ 1.4	7.5 $\pm$ 1.3	6.8 $\pm$ 1.9	8.0 $\pm$ 2.1
		female 5.0 $\pm$ 1.2	4.6 $\pm$ 1.7	4.3 $\pm$ 0.7	7.1 $\pm$ 3.2	4.9 $\pm$ 1.0	7.2 $\pm$ 2.7

Figure 15: Design of short-term fasting experiments

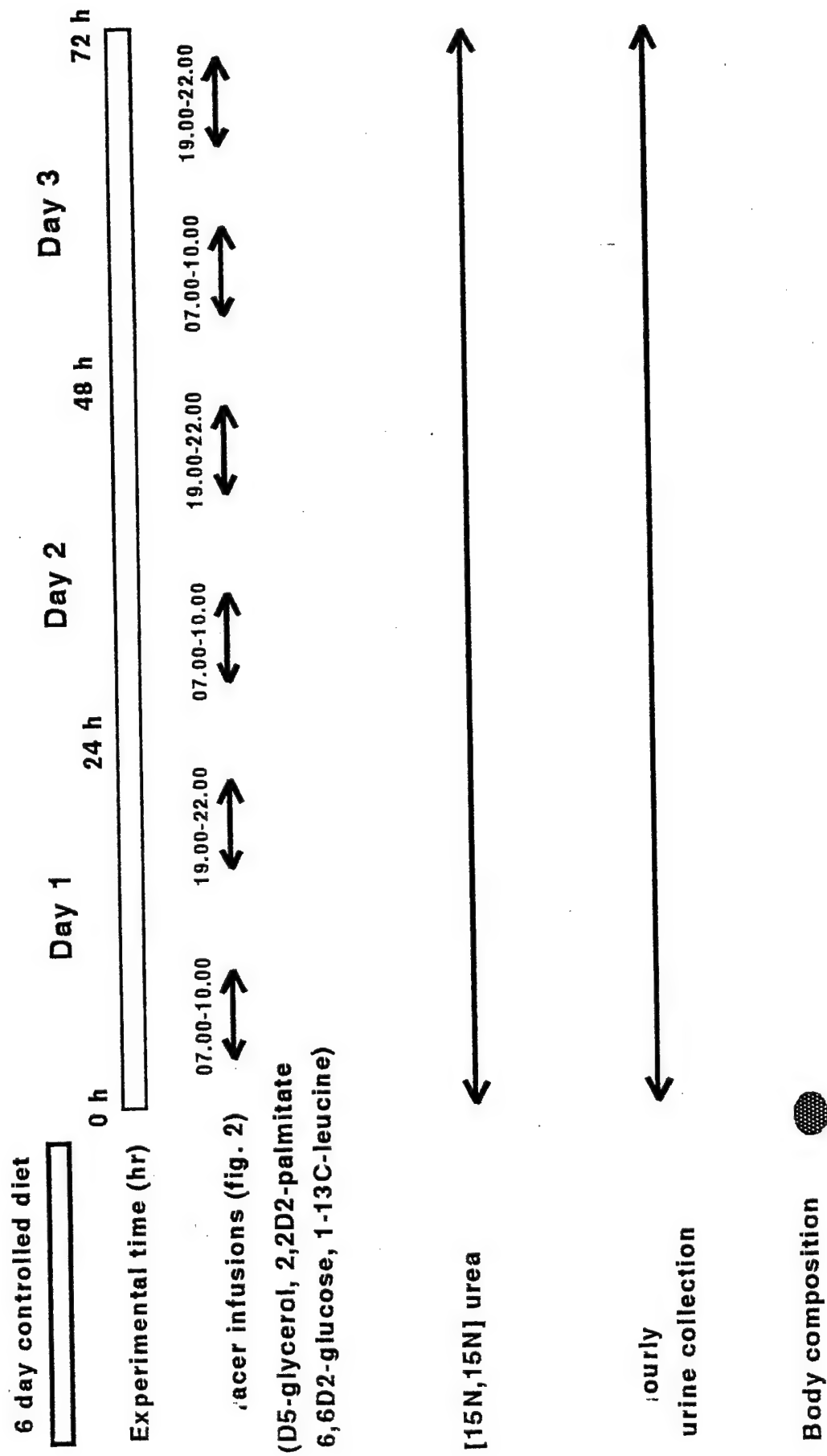


Figure 2S

# Tracer infusion protocol for short-term fast (Series I)

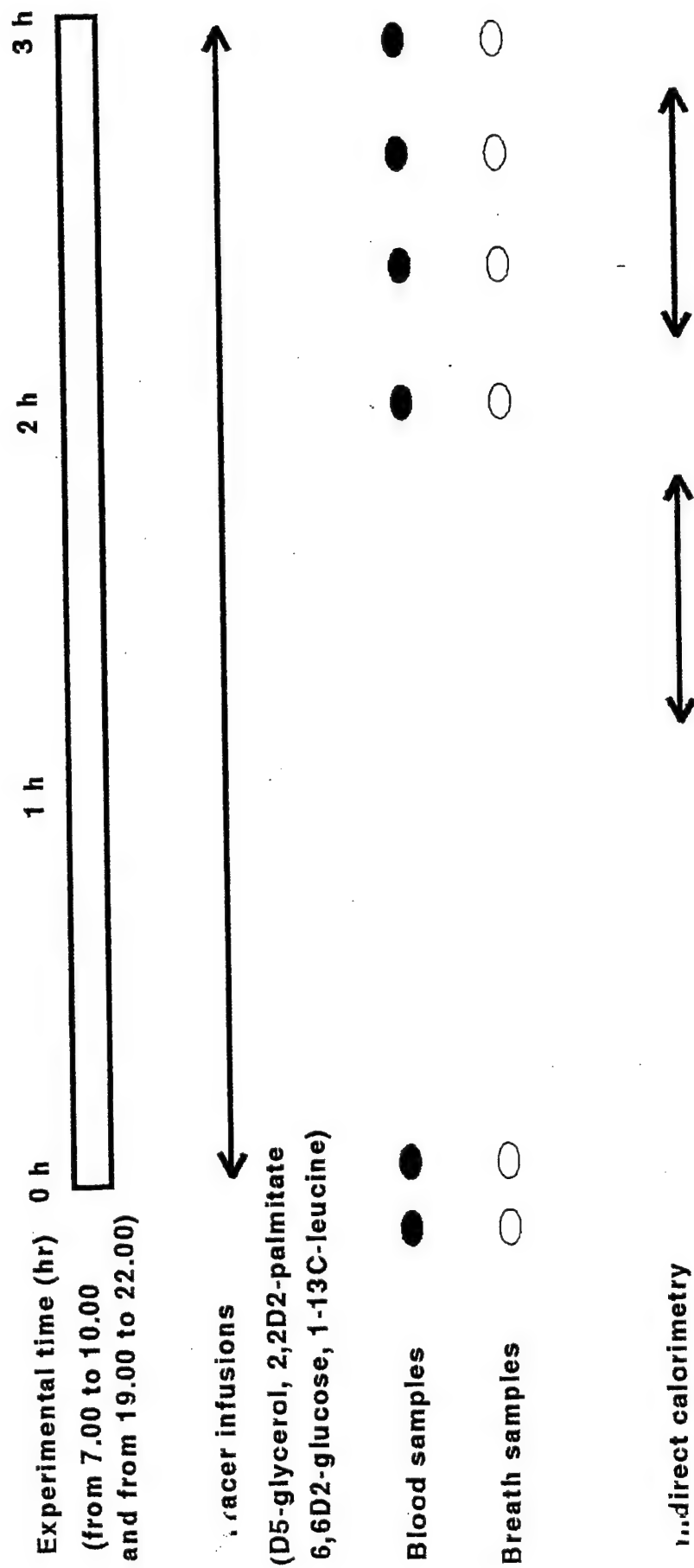


Figure 3S

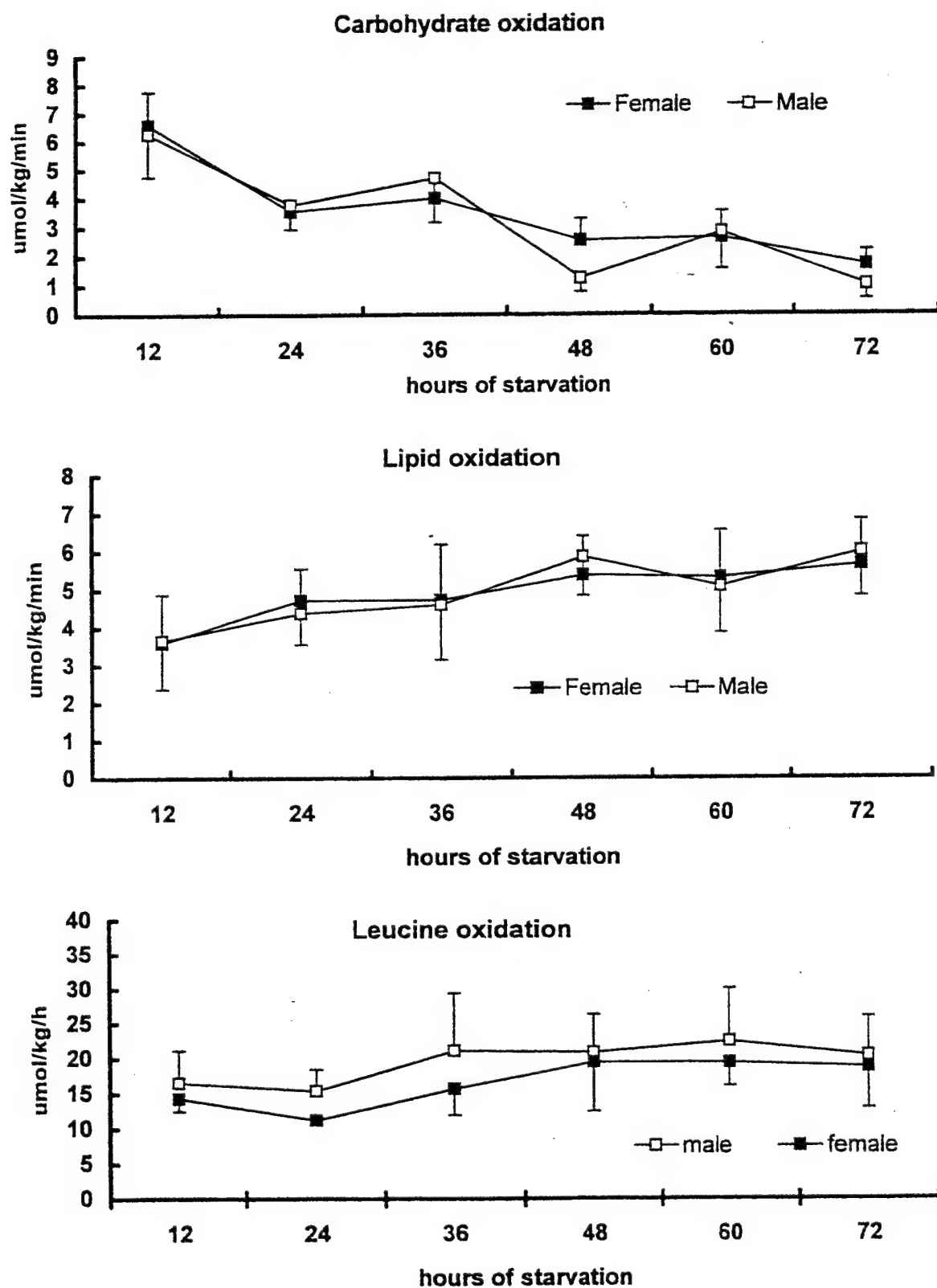


Figure 4S

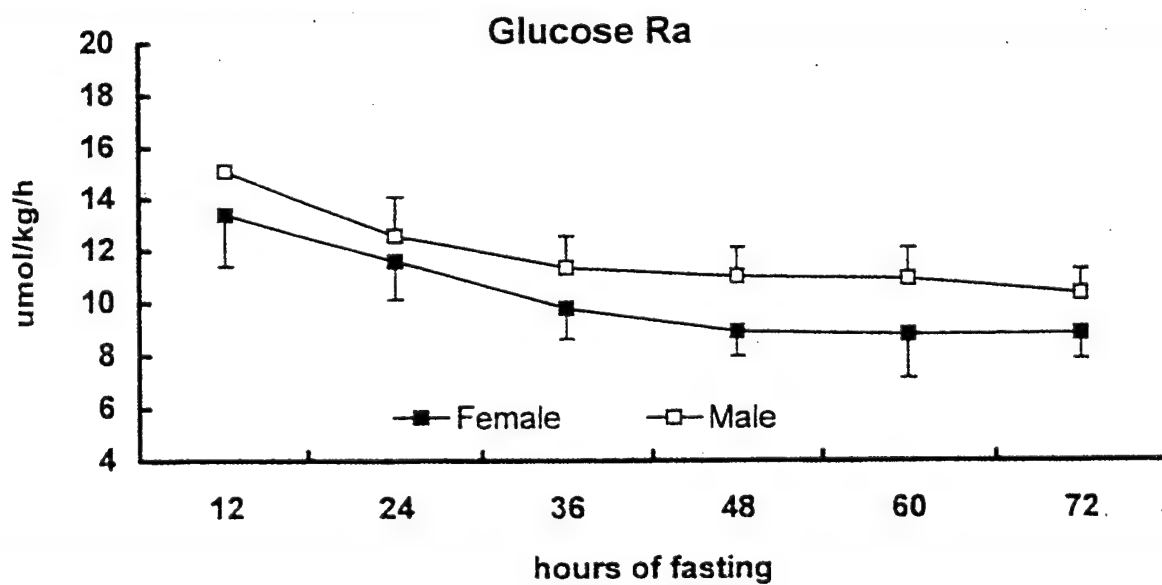
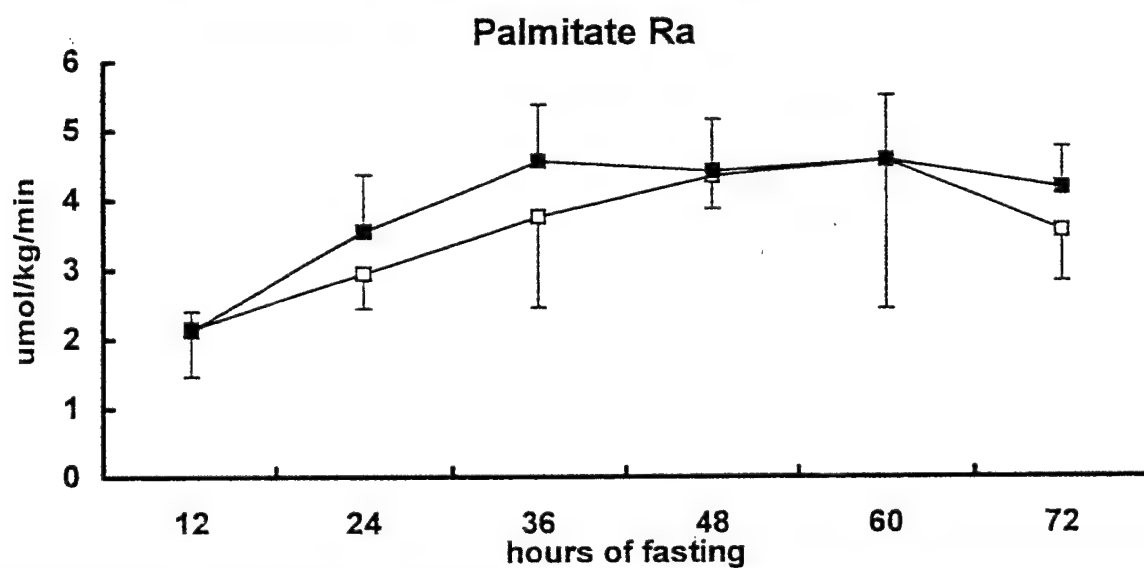
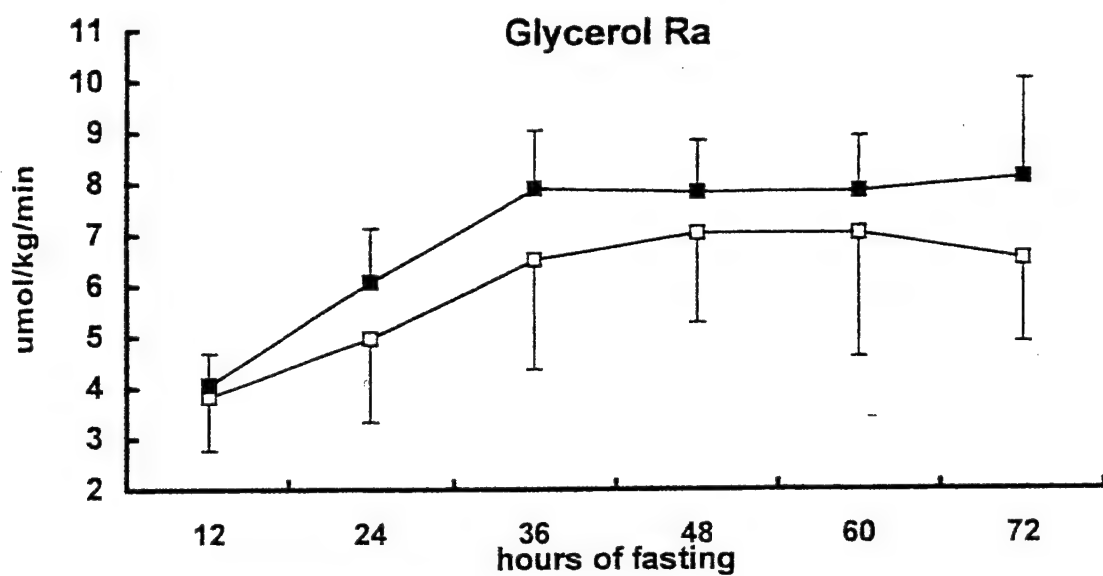


Figure 5S

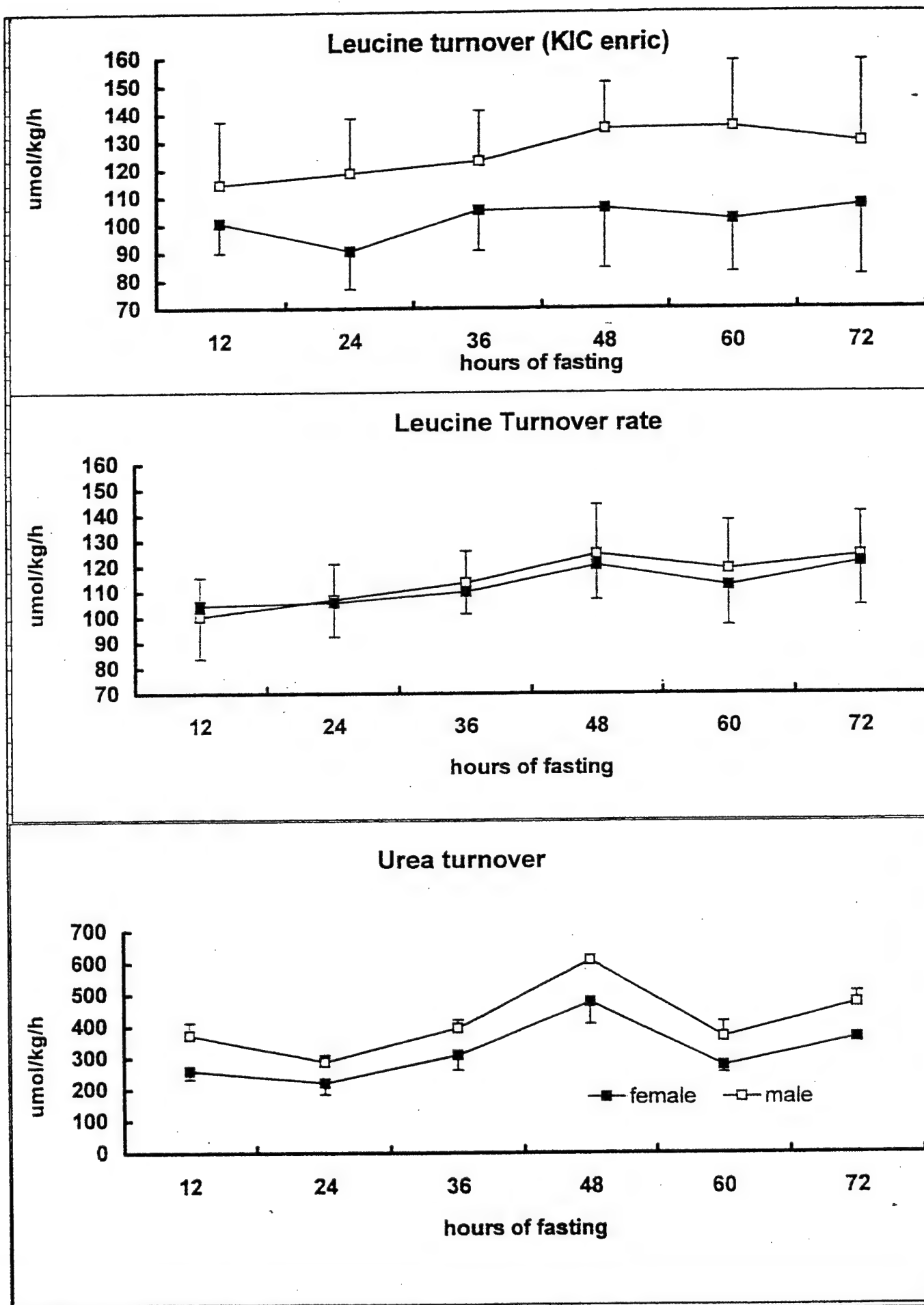


Figure 6S

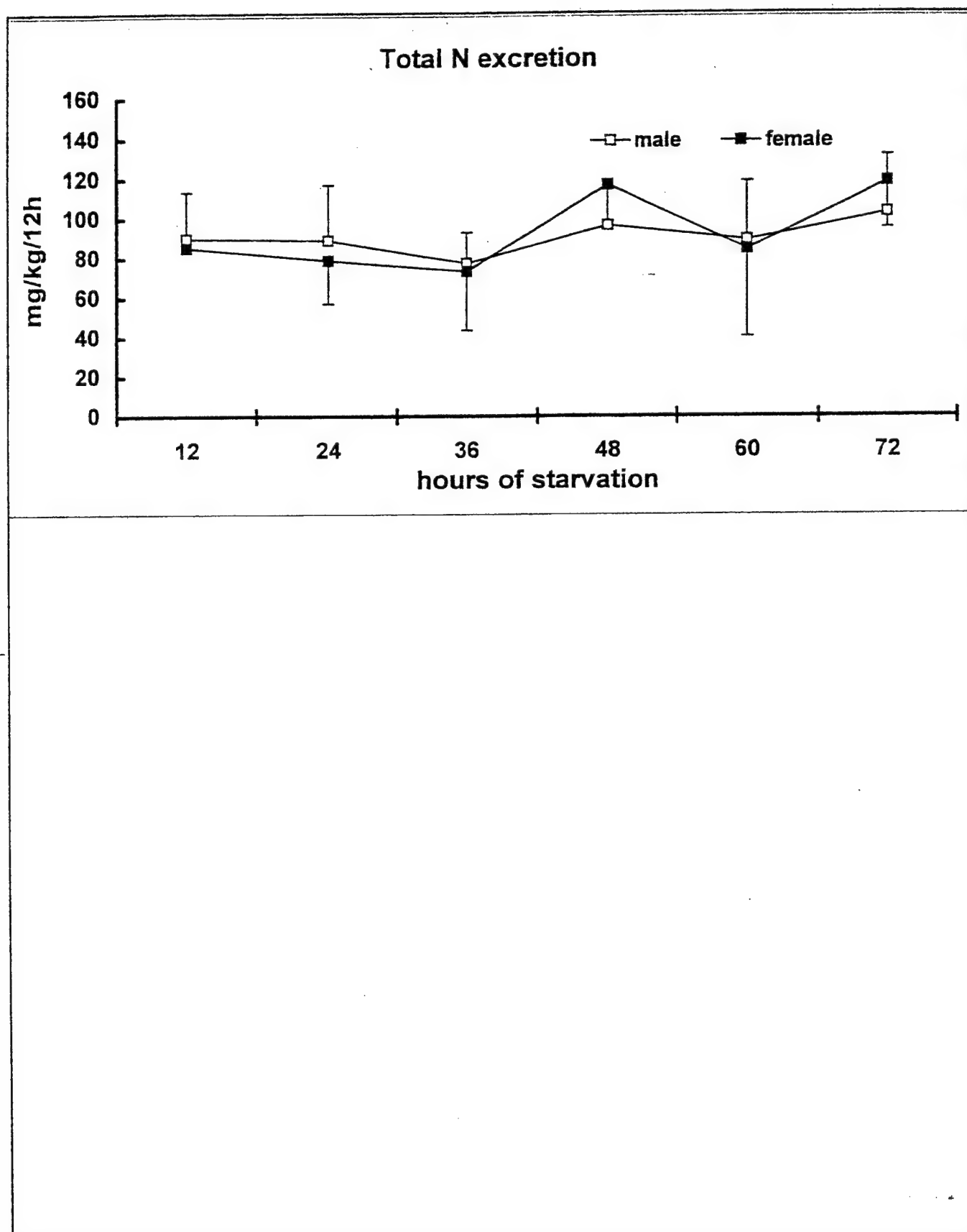


Figure 1L

## Design of hypocaloric study

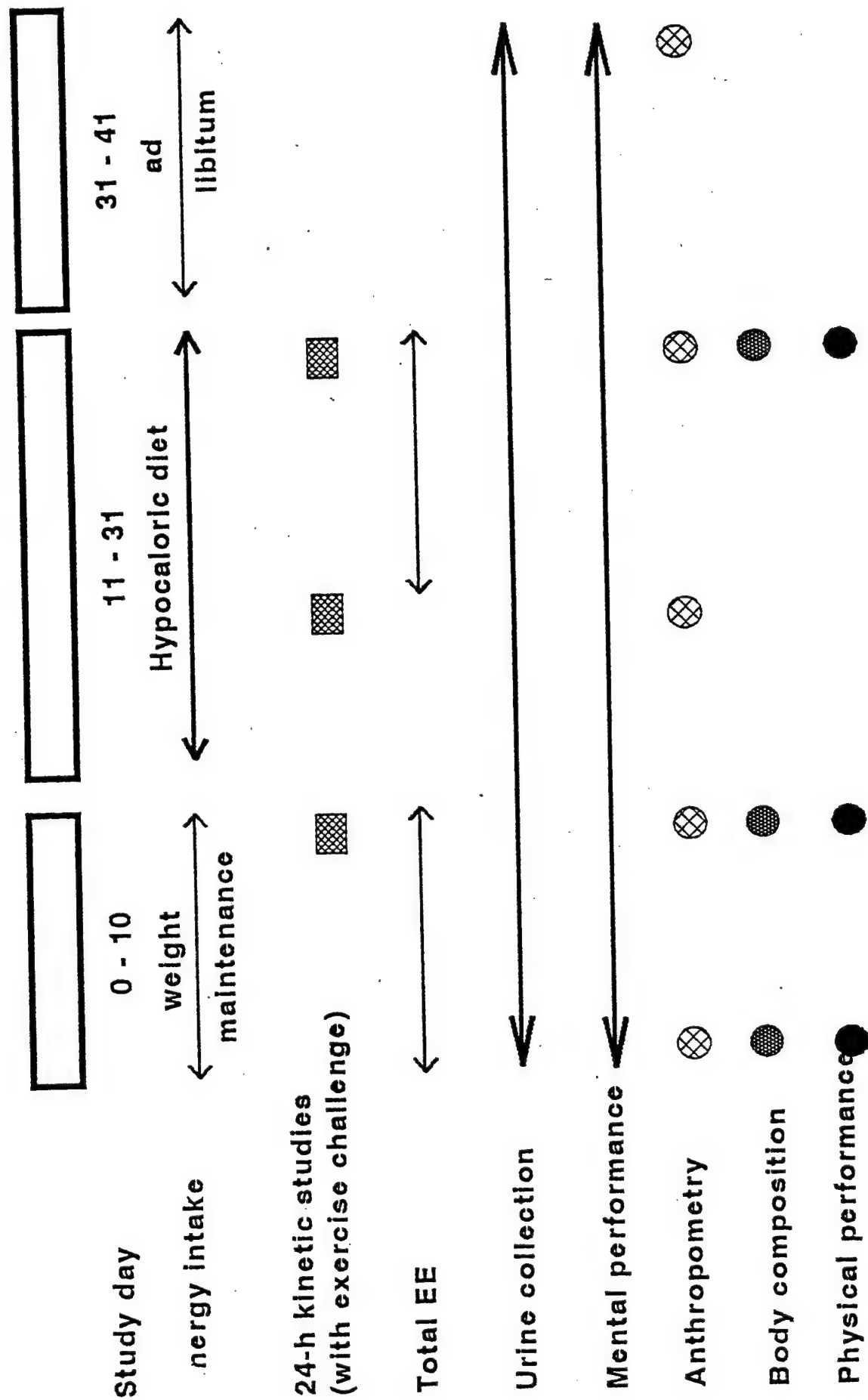


Figure 2L

# Tracer infusion protocol (24 h study)

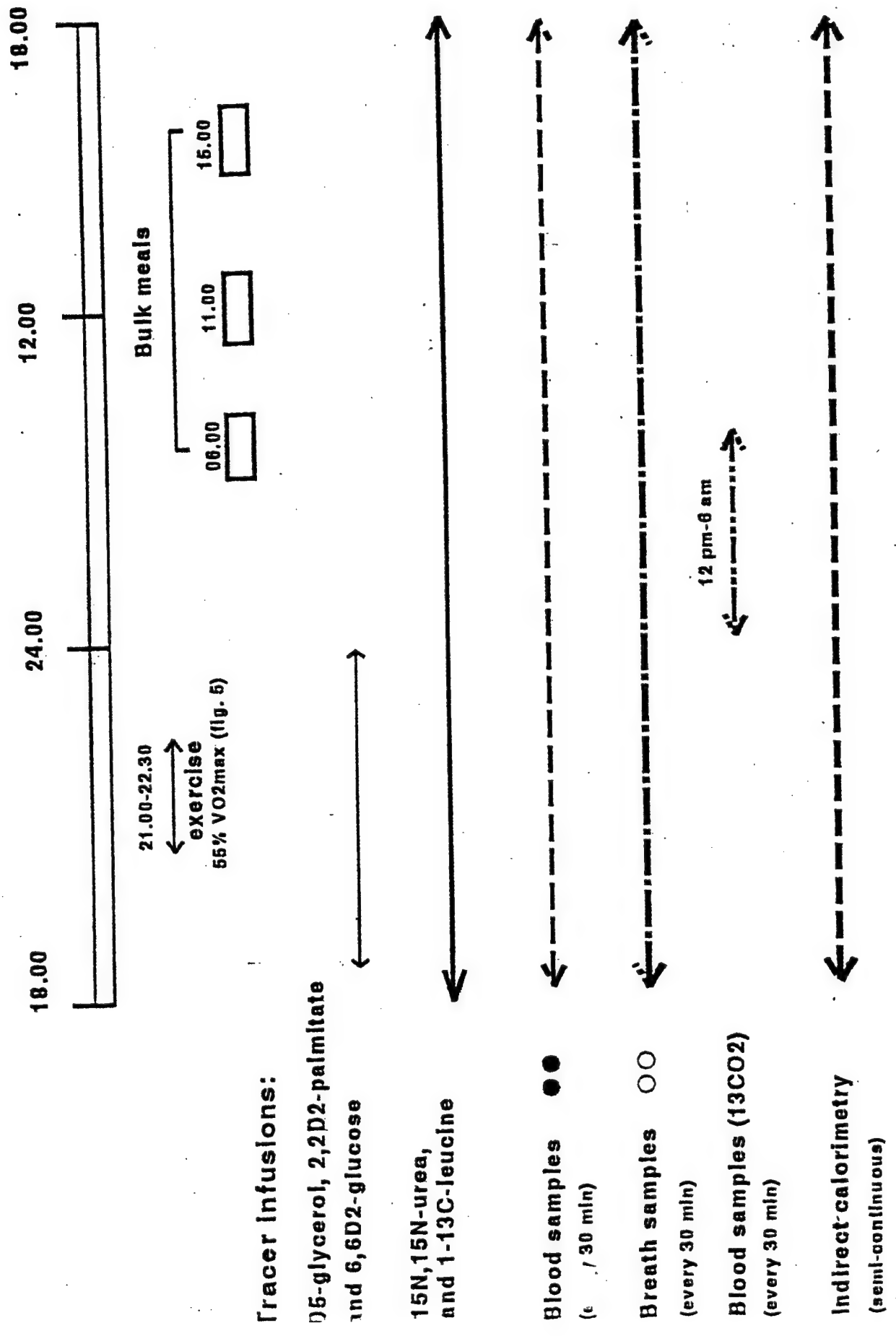
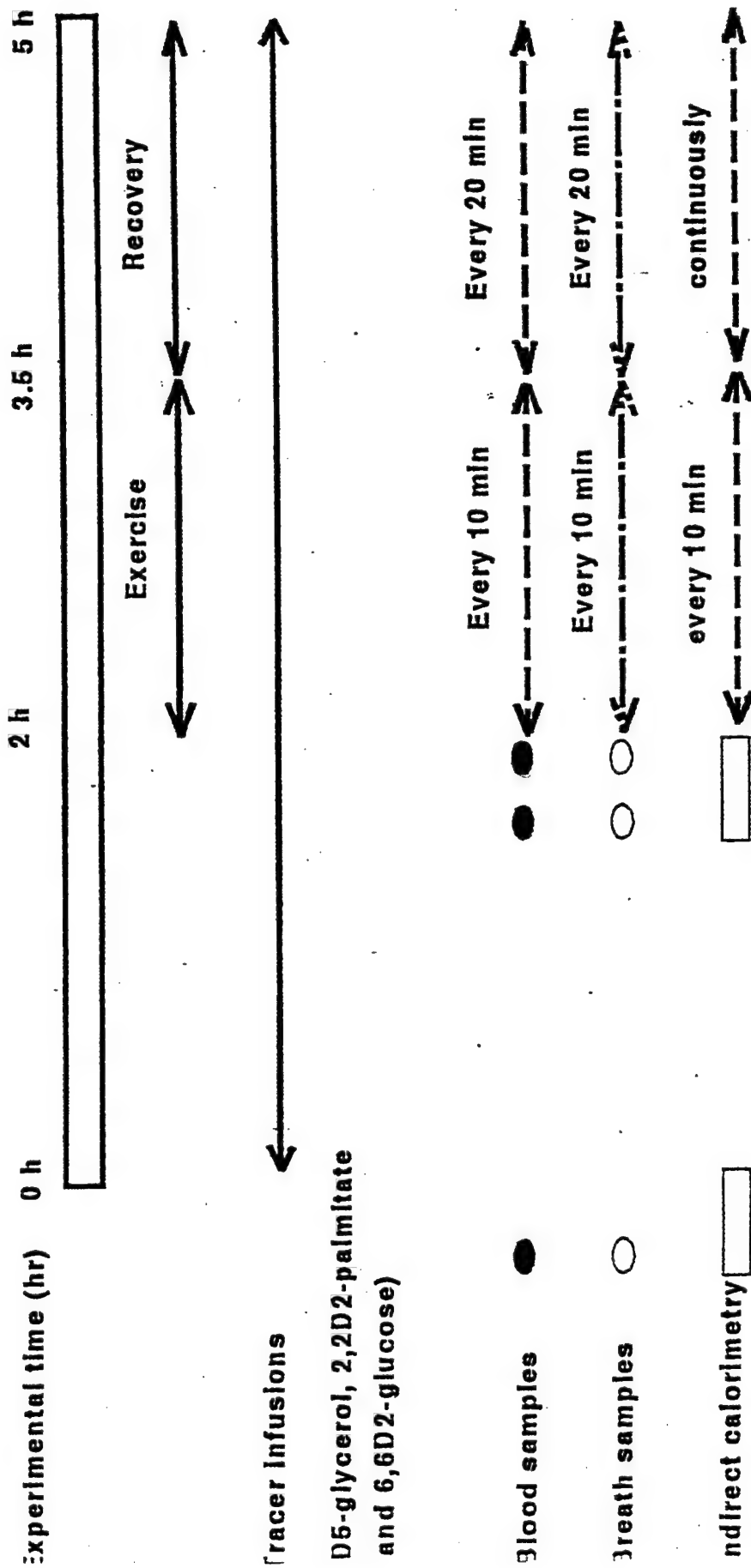
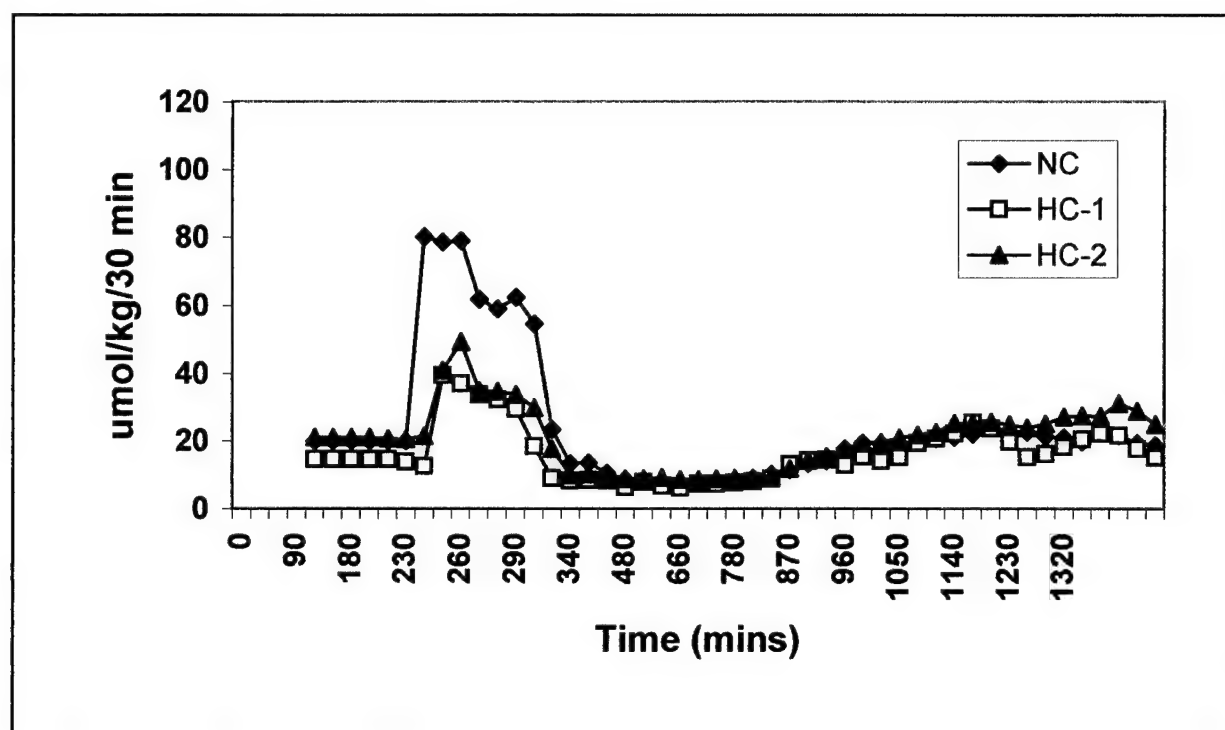


Figure 3L

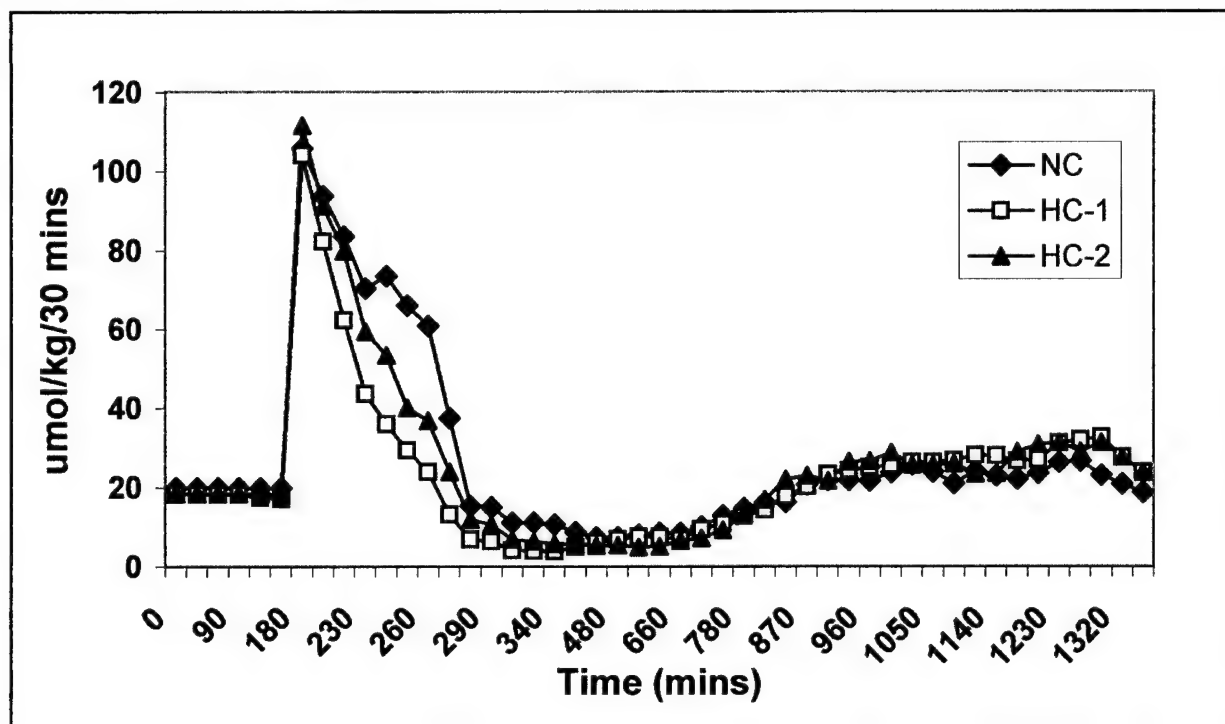
# Tracer infusion protocol: Exercise-challenge



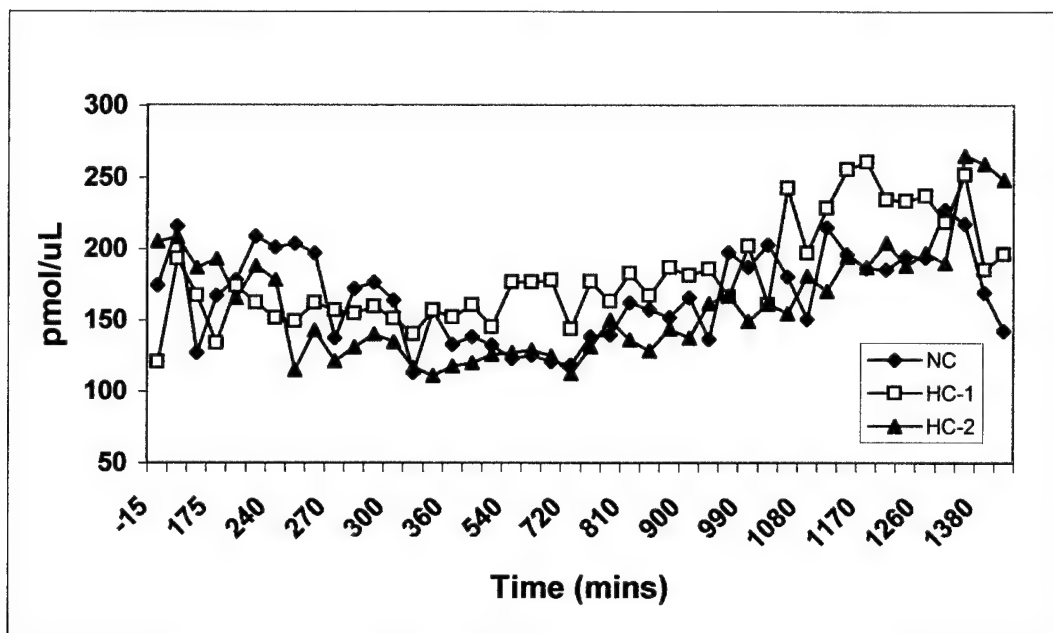
**Figure 4L: 24h pattern of leucine oxidation in male subjects studied over normocaloric and hypocaloric intakes**



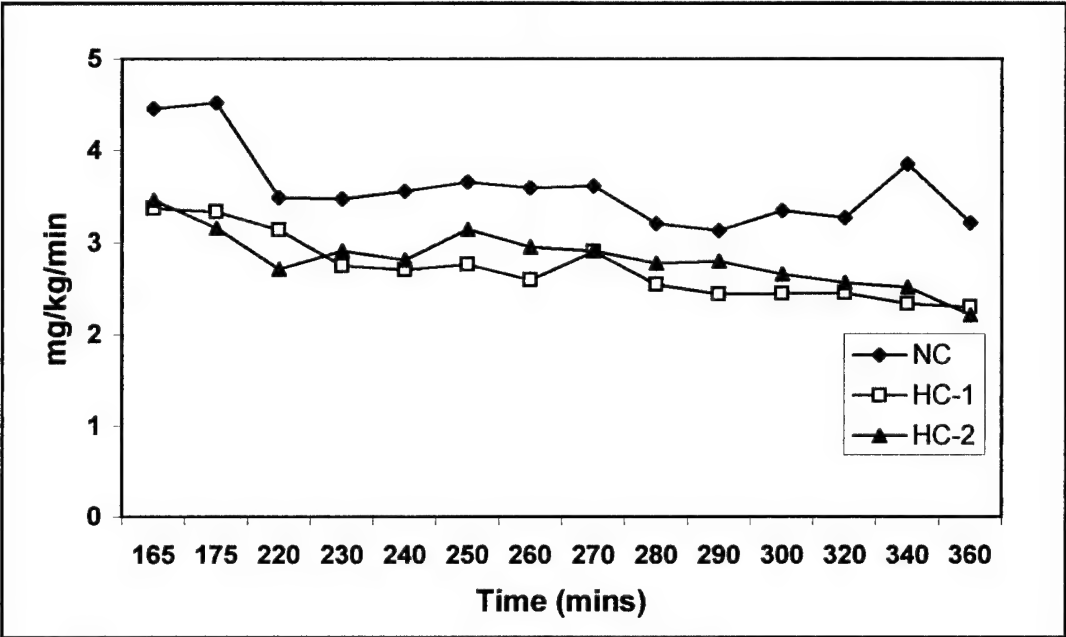
**Figure 5L: 24h pattern of leucine oxidation in female subjects studied over normocaloric and hypocaloric intakes**



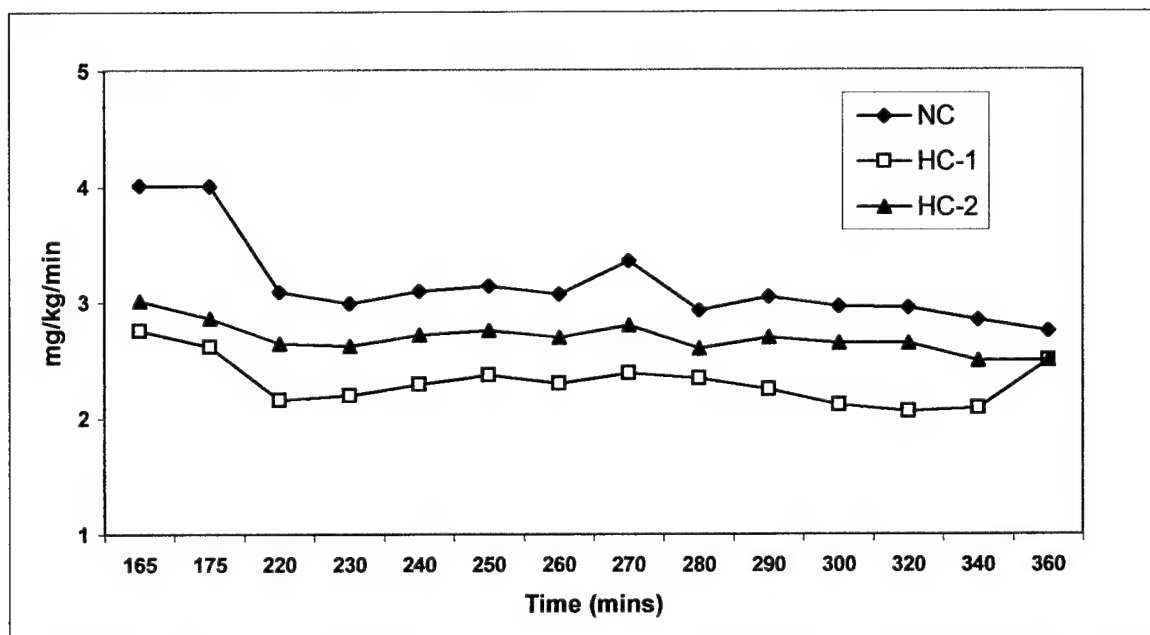
**Figure 6L: Plasma leucine concentrations in male and female subjects studied over normocaloric and hypocaloric intakes**



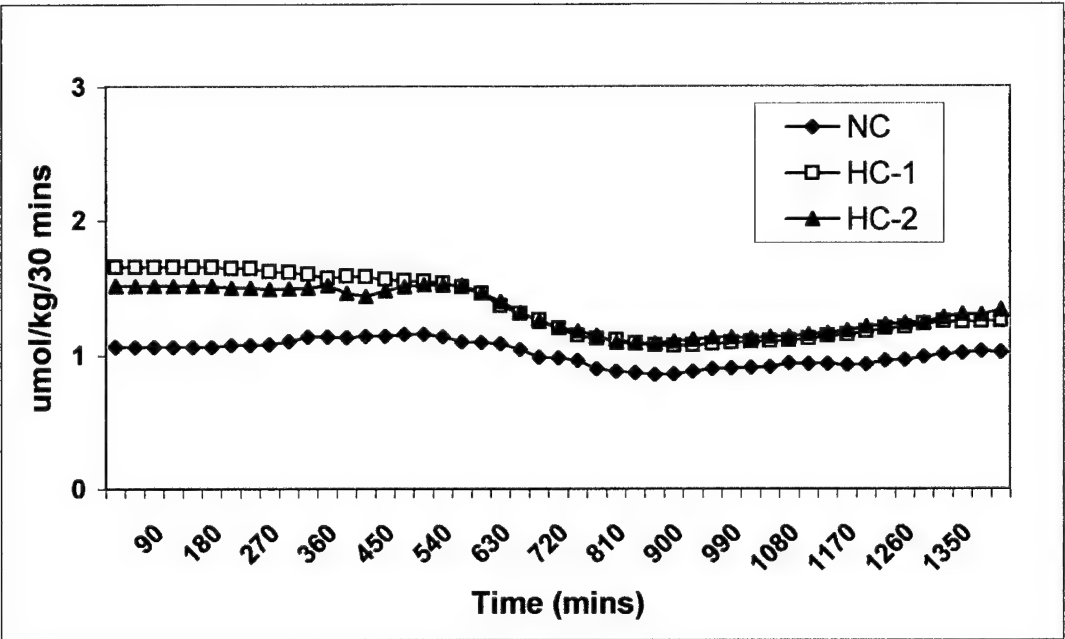
**Figure 7L: Ra of Glucose in male subjects studied over normocaloric and hypocaloric intakes**



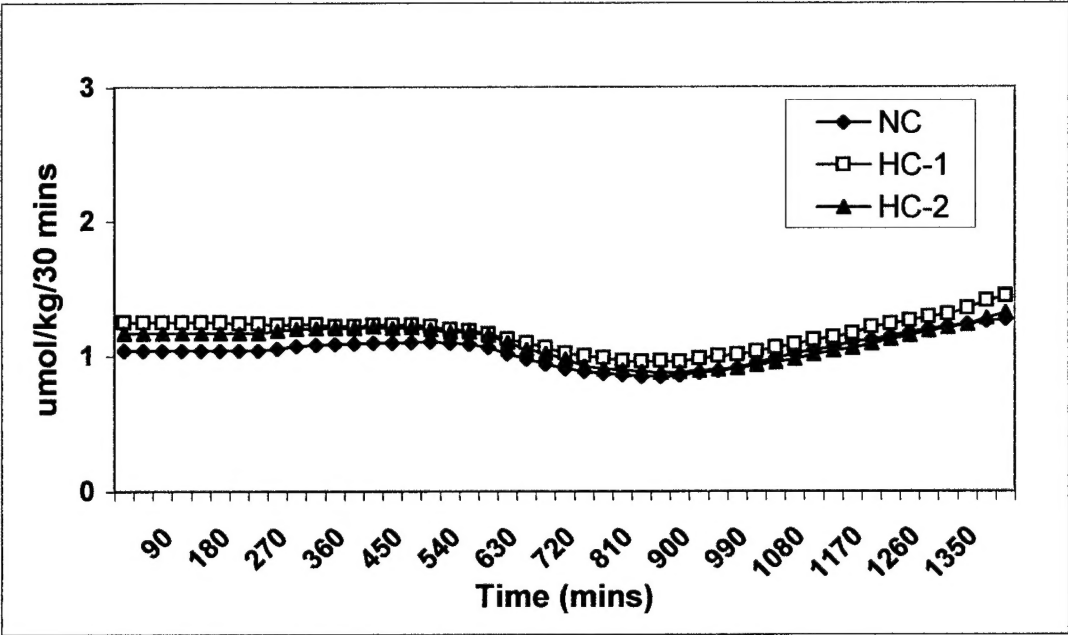
**Figure 8L: Ra of glucose in female subjects studied over normocaloric and hypocaloric intakes**



**Figure 9L: 24h pattern of Urea production in male subjects studied over normocaloric and hypocaloric intakes**



**Figure 10L: 24h pattern of Urea production in female subjects studied over normocaloric and hypocaloric intakes**



## FOREWORD

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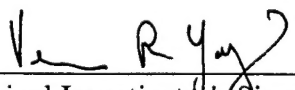
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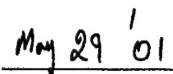
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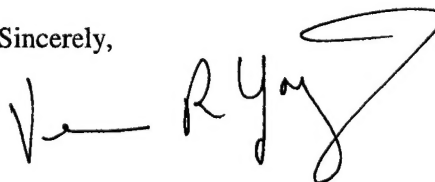
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# **FINAL REPORT**

**DAMD 17-95-2-5104**

**May 2001**

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